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STRAWBERRY BREEDING STUDIES INVOLVING CROSSES BETWEEN THE CULTIVATED VARIETIES (× FRAGARIA ANANASSA) AND THE NATIVE ROCKY MOUNTAIN STRAWBERRY (F. OVALIS) [1]

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INTRODUCTION

The strawberry is perhaps the most widely grown of all the fruits in the United States; it is not only particularly adapted to home gardens but is also a highly important fruit of commerce. However the strawberry plant is too tender to survive and produce satisfactorily without winter protection in almost all of the northern half of the United States, especially in the northern Great Plains areas where winters are very severe. Consequently, the development of hardier varieties should reduce the cost of growing strawberries where winter protection is now necessary.

The value of the native Rocky Mountain strawberry (*Fragaria ovalis* (Lehm.) Rydb.) in breeding more desirable varieties of cultivated strawberries (× *F. ananassa* Duch.) has been pointed out by Georgeson (*7*),[3] Darrow (*2*), and Hildreth and Powers (*9*). It should be noted, however, that these workers used the species names *platypetala* and *cuneifolia* instead of *ovalis*. Crosses between cultivated varieties and collections of *F. ovalis* have been grown at the United States Department of Agriculture Cheyenne Horticultural Field Station, Cheyenne, Wyo., for a number of years. This paper reports the progress being made there in breeding strawberries, especially that dealing with characters of economic importance, and it evaluates the breeding methods used. The immediate objective of the breeding program is to recombine the large size of fruit of the commercial varieties with the winter hardiness of the native Rocky Mountain strawberry.

REVIEW OF LITERATURE

The literature dealing with strawberry breeding is rather extensive, and only that part having a direct bearing on the present study will be reviewed here. The reader is referred to Bunyard (*1*) for a more complete discussion of the history and development of the strawberry and to Darrow (*2*) for a review of the literature dealing with breeding.

Bunyard (*1*) pointed out that even though the wild strawberries, including *Fragaria virginiana*, had been grown as garden plants for some 300 years before the introduction of *F. chiloensis* into France by

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[3] Italic numbers in parentheses refer to Literature Cited, p. 122.

Frezier, no improved variety had been recorded. His following remarks are particularly interesting:

> We see, therefore, that *F. virginiana*, left merely to the stimulus of cultivation, did not produce any varieties of special size or remarkable in other qualities, and it was generally admitted that for flavour the original type was unsurpassed. Nothing at all approaching in size the Strawberry of modern times had been produced, and the reason of this was, doubtless, that no character giving large fruit had been introduced into the gametic constitution of existing fruits.
>
> The introduction of the Chilian Strawberry, *F. chiloensis*, brought, however, the required size into combination with the flavour of the Virginian, and thus laid the foundation of the fruit as we know it to-day. The introduction of the Chilian Strawberry was, therefore, an event of the first importance in Strawberry history . . .

Hence, it seems that the modern cultivated strawberry resulted from crosses involving *F. virginiana* and *F. chiloensis*. This conclusion is in accord with that of Darrow (*2*).

The literature dealing with the use of *Fragaria ovalis* to improve the cultivated strawberry is limited. Georgeson (*7*), working in Alaska, used *F. chiloensis*, *F. ovalis*, and the cultivated strawberry to develop a berry suitable for Alaskan conditions. He succeeded in producing strawberries suitable for the interior of Alaska, where the temperature often falls as low as 50° F. below zero and sometimes as low as 65° below. The following statement by Georgeson (*7*, *p. 8*) sets forth the merits of one of his hybrids:

> One of the best hybrids so far produced has resulted from crossing the Magoon, a well-known strawberry of the Pacific Coast, with pollen from the wild plant of the interior. This hybrid, so far as tested, possesses all the desired qualities. The berry is large, of deep red color not merely on the surface but throughout, and it is firm enough to stand shipment. The berry has a good flavor and a calyx that separates easily when the fruit is ripe.

Darrow (*2*) pointed out the value of *Fragaria ovalis* as a source of cold and drought resistance, everbearing types, and earliness.

Hildreth and Powers (*9*) reported the results from tests involving extensive collections of the Rocky Mountain strawberry. In 1936 systematic collections were made under the supervision of A. C. Hildreth. About 42,000 plants were collected from more than 1,100 localities in Wyoming, Colorado, Montana, New Mexico, and Utah. None of these had fruits large enough for commercial production, although they were generally of high quality. As grown in the trial plots, these native plants show variation in many horticultural characters, differing in size, shape, color, and quality of fruit and in season of maturity, fruiting habit, prolificacy, tendency to produce runners, resistance to disease, and tolerance to soil alkalinity. Some are distinctly everbearing, others are June bearers. Some show considerable drought resistance, and the majority of them have proved hardy without protection at Cheyenne, Wyo. The F_1 plants from crosses between cultivated varieties and different collections of the native strawberry were found to vary within and between crosses as regards the following horticultural characteristics: Size, sweetness, flavor, aroma, shape, color, prolificacy, and time of maturity of fruit; June or everbearing habit; position of fruits on the plant; type of flower cluster; shape, size, and abundance of leaves; and habit of plant growth.

Another problem in the breeding of *Fragaria* arises from the fact that some supposedly F_1 hybrids resemble the maternal parent and

others resemble the paternal parent. Such cases have been reported by Millardet (*11*), East (*4, 5*), Ichijima (*10*), and Rygg and Darrow (*15*). Ichijima described both maternal and paternal types of inheritance. In the case of maternal inheritance the chromosome number was that of the female parent, and in case of paternal, that of the male parent. All these populations of matroclinous type show no trace of hybridity in their genoms. On the other hand, the hybrid nature of the genoms never failed to show in those populations that exhibit their hybrid character morphologically. The exact method of occurrence of these unusual types is not known, but it is evident that among the F_1 populations there occur individuals which possess the nuclear complex of one or the other parent. The possibility of the occurrence of these exceptional individuals must be taken into account in breeding or genetic studies involving *Fragaria*.

EXPERIMENTAL CONSIDERATIONS

MATERIAL

Since the mode of reproduction in the cultivated strawberry is asexual, only one plant combining the desired characters in any segregating population is necessary for the production of a variety possessing the traits sought. Most strawberry varieties have perfect flowers, but a few have pistillate flowers only. Varieties having perfect flowers may be cross-pollinated by insects, and in hybridizing strawberries precautions must be taken to prevent off pollinations.

The results of a study of the behavior of the chromosomes during meiosis in the parents and in the crosses between cultivated varieties and the Rocky Mountain strawberry have already been reported (*14*). Chromosome behavior during meiosis of the parents and F_1 hybrids was found to be essentially normal. However, some irregularities similar to those found during meiosis of *Triticum* (*12*) do occur.

Because of a lack of sufficient breeding, genetic, and karyological information for crosses involving the cultivated varieties and the native Rocky Mountain strawberry, selection of the parents and the methods of breeding were based upon the phenotypic characters of the varieties and collections. The variety Gem was used in crosses because of its large fruit under the environmental conditions prevailing at Cheyenne and its everbearing habit, and because it comes nearer to being winter hardy than any of the other cultivated varieties tested. However, the fruit of Gem is lacking in quality. Of the commercial varieties of high quality tested by Hildreth at the Cheyenne Horticultural Field Station, Fairfax and Dorsett came the nearest to being winter hardy. Therefore, they were selected along with Gem as the cultivated varieties to be used in the strawberry-breeding program.

When collections of the Rocky Mountain strawberry were being selected for crossing, winter hardiness was given the first consideration. Since most of the collections of *Fragaria ovalis* possessed this trait (*9*), selection for other desirable characteristics was made easier. Collections as genetically diverse as possible were sought. Without the aid of intercrosses and progeny tests to determine genetic disparity, phenotypic differences were used for making the selections. The collections having winter hardiness and meeting these requirements were 36979, 361477, and 37501. In addition, 36979 had high quality

and was very prolific and 37501 bore larger fruit than the average collection of the Rocky Mountain strawberry and also had fair quality.

In analyzing and interpreting the experimental data it should be kept in mind that all of these parents may be heterozygous for at least some characters and that all plants of a certain collection are not necessarily alike genetically.

EXPERIMENTAL DESIGN

The experiment consisted of two parts: That dealing with breeding methods based upon fundamental genetic principles and that dealing with methods of conducting the experiment based primarily upon the fundamentals of biometry.

Methods of breeding in which hybridization is utilized may be broadly classified into two categories. The first is inbreeding followed by hybridization, which is particularly suited for breeding crops that are not self-pollinated and in which heterosis of some characters is of economic importance. It is employed when the available material is both highly heterozygous and heterogeneous and does not contain lines, strains, varieties, or species possessing sufficient genetic disparity for heterosis in the crosses to be of economic importance. The second is hybridization before inbreeding, which is used with crops in which lines, strains, varieties, or species possessing distinct genetic disparity do exist.

Since the commercial varieties of strawberries possess large fruits and are not winter hardy as compared with the native Rocky Mountain strawberry, and the latter possesses small fruits and as a rule is extremely winter hardy, it would seem that the primary objective of the breeding program would be attained most easily and rapidly by hybridizing before inbreeding.

Most F_1 hybrids originating from parents as diverse phenotypically as the cultivated varieties of strawberries and the native Rocky Mountain strawberry show decided heterosis of the vegetative parts of the plant. The problem, therefore, was to find a method of handling the hybrid material that would allow segregation of the genes differentiating size of fruit and winter hardiness and still maintain heterosis of the vegetative parts. The solution was found in the use of double crosses. For a better understanding of the problem the parental material is grouped below on the basis of the characters that are to be given primary consideration:

Non-winter-hardy varieties with large fruit (×*Fragaria ananassa*): Fairfax, Dorsett, Gem.
Winter-hardy collections with small fruit (*F. ovalis*): 36979, 37501, 361477.

If *a* stands for the cultivated varieties and *o* for the collections of *Fragaria ovalis*, the possible ways of making the double crosses are as follows: $F_1(a\times a)\times F_1(o\times o)$ and $F_1(a\times o)\times F_1(a\times o)$. If the double cross is made in the manner first indicated, there will be a minimum of segregation of the genes differentiating degrees of winter hardiness, size of fruit, and vegetative vigor, since the greatest genetic disparity as judged by the differences between phenotypes would be expected to occur between the two groups *a* and *o*. However, if the cross is made according to the second method there will be a maximum of segregation of the genes differentiating winter hardiness, size of fruit, and vegetative vigor. This is just what is desired, as it provides

greater possibilities for obtaining plants combining winter hardiness and large size of fruit. However, such is not the case with vegetative vigor because, if the original F_1 plants exhibited heterosis and if the genetic disparity between the commercial varieties and that between the collections of the Rocky Mountain strawberry were negligible, then the double crosses made in the second manner should show a decided decrease in heterosis or vegetative vigor of plants. The double crosses actually made were $F_1(a \times o) \times F_1(a \times o)$, because this allowed a maximum segregation of the genes differentiating size of fruit and winter hardiness.

The design of the experiment was similar to that described in an earlier paper (*13*), and since the reasons for including the different populations have already been given, they need not be repeated here. The experimental design was a randomized complete block for the seed (achene) germination and the field studies. The number of blocks for the field studies was 10 and the number of plants per plot was 12. Since an inadequate number of plants was obtained for the F_1 population of Fairfax × 37501 and the selfed progeny of Fairfax, these populations were included in the first 6 replications only. In all comparisons and deductions including these populations, this fact was taken into account.

The symbol D. C. is used to designate a double cross involving two F_1 hybrids; S_1 is used to designate the progeny obtained by self-pollinating the parent indicated; and B_1 is used to designate the progeny obtained by backcrossing the F_1 hybrid Fairfax × 36979 to the parent indicated.

The populations in the breeding program, together with the number of plots grown per block for the field studies, are as follows:

Population:	*Plots per block (number)*
Fragaria ovalis:	
37501 (asexual)	1
361477 (asexual)	1
36979 (asexual)	1
36979 (S_1)	1
Hybrid populations:	
B_1[F_1(Fairfax × 36979) × 36979]	1
F_1(Fairfax × 36979)	1
F_1(Fairfax × 361477)	1
F_1(Fairfax × 37501)	1
F_2(Fairfax × 36979)	3
D. C. [F_1(Fairfax × 36979) × F_1(Dorsett × 37501)]	4
D. C. [F_1 (Fairfax × 36979) × F_1(Gem × 361477)]	3
B_1 [Fairfax × F_1(Fairfax × 36979)]	3
× *F. ananassa:*	
Fairfax (S_1)	1
Fairfax (asexual)	1
Gem (asexual)	1
Dorsett (asexual)	1

The original purpose of the study of seed germination was to determine whether there were differences in percentage and time of germination between populations and within populations and the relative importance of possible differences. As the investigation progressed an attempt was also made to determine the effect of the female parent upon the percentage and time of germination.

Since another objective was added after the experiment was started, the investigations consisted of the 2 rather distinct parts just men-

tioned. As regards the first part of the germination studies, the variates were the same as those for the field studies except that, of necessity, those asexually propagated were omitted and the number of plots per block differed for some of the populations. These differences were as follows. There were 4 plots per block of the F_2 population, 5 per block of the double cross involving Dorsett, and 5 per block of the backcross to Fairfax. The number of blocks for these seed germination studies was 17 and the number of seeds per plot was 25, whereas the number of blocks for the second germination study was 20 and the number of seeds per population was 500. The seeds were placed between moist blotters in the germinator on February 12, 1940, and August 22, 1941, respectively, and held for 140 days at an average temperature of 30° C. The seeds were approximately 1 year and 2½ years old, respectively. The greatest range in temperature was from 28° to 32°. The young plants were kept in the blotters until they developed roots and cotyledons and then were transferred to 2-inch pots. This allowed the recording of abnormal seedlings. Since such seedlings were so rare as to be negligible in these germination studies, they will not be considered further. The number of germinated seeds was recorded daily. However, in reducing the data they were classified into 5-day periods. For example, the first period for the seeds put in the germinators on February 12, 1940, is 40 (see table 1), and the frequencies given represent the number of seeds which germinated 38 to 42 days after the seeds were placed in the germinators.

CHARACTERS

In studying biological characters it is desirable whenever possible to record the results in standard units. However, because of the nature of the character or the time and labor involved in employing standard units, it is sometimes necessary to resort to observation. In the present work it was possible to measure the following characters by standard units: Germination (seeds germinated), plant height (centimeters), length of period from May 1 to first bloom (days), length of period from first bloom to first fruit ripe (days), and length of period from May 1 to first fruit ripe (days).

For all characters for which grades were used the numerical values were 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5. To establish these grades the range in expression of the character for the parents and F_1 hybrids was first determined. Then tentative grades of 1, 2, 3, 4, and 5 were set up and these in turn were used to establish the fractional grades. With these tentative grades in mind the classification of the experimental material was started. Usually after the classification of two or three replicates any necessary adjustment of grades had been made and the grades had become fixed in mind. Then the classification was made of all replicates. During the classification of some characters it was found that the work was expedited by having samples of the middle grades (1.5, 3.0, and 4.5) available for making comparisons.

The notes on winter hardiness were taken on May 8, 1941, and were checked on June 3. All the plants in a hill (plants derived from one original plant by asexual propagation) receiving a grade of 0.5 were entirely free from any discernible injury. A grade of 1.5 was given to hills that had a few plants showing slight injury. Grades from 3.0 to 5.5, inclusive, were given to hills in which all the plants showed some

crown injury. Grades of 4.5 and above were given to hills the plants of which looked as if they would not survive even though some growth had started on May 8. By June 3 all the plants of most of those receiving grades of 4.5 or higher were dead. Hills having all plants dead on May 8 were given a grade of 5.5. The grades not specifically mentioned represent degrees of winter injury falling between those discussed.

Grades for size of fruit were established as follows. After the F_1 hybrids and parental varieties of the first two replicates had been examined, fruits having the following greatest diameters were taken as the standard for the grades designated:

Grade:	*Diameter (inches)*
1	5/16
2	8/16
3	12/16
4	1.0
5	1¼

The fractional grades, with the exception of 0.5 and 5.5, fell between the indicated measurements. The value 0.5 was assigned to all fruits smaller than grade 1 and 5.5 to all fruits larger than grade 5.0.

The plants per hill were counted for the first 5 replicates to determine the grades for runner plants. Then, on September 19 and 20, 1940, the plants of each replicate were classified. As these notes were taken before the hills derived from a single plant had been trimmed to occupy a 15- by 15-inch space, the grades would be expected to record the ability of the seedling or asexually propagated plant to produce runners. To determine whether such actually was the case, a correlation coefficient which involved the grades for each plant of the first 5 replicates and the number of plants per hill was calculated. The correlation coefficient was +0.853, which shows that the grades are a reliable estimate of the ability of the original plants to produce runners. By calculating the regression equation, it was found that the number of plants per hill increased 15.2 for each increase of 1 grade.

Sweetness, flavor, and firmness were ascertained by tasting fruits of each plant. The grades for flavor were based on the intensity of the strawberry flavor relished by most people. During the taking of this note it was observed that the intensity of flavor seemed to be positively associated with the higher degrees of sweetness.

EXPERIMENTAL RESULTS

The experimental results are divided into two parts, one dealing with the germination studies and the other with the characters taken in the field.

GERMINATION STUDIES

The data on amount and time of germination are given in table 1, and the χ^2 values for testing the statistical significance of the differences between populations as regards both the ratio between germinated and nongerminated seeds and the time of germination are given in table 2. These values are the heterogeneity χ^2's. The method of partitioning the data and the formulas used were given in an earlier paper (*14*) and need not be repeated here. In calculating the χ^2 values the data were grouped so that the least number of individuals expected in any one category was five or more (see Fisher *6*).

TABLE 1.—*Frequency distributions of germinating strawberry seeds approximately 1 year old*

Population	Seeds germinating after being in the germinator for the indicated number of days																				Total germinating
	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Fragaria ovalis (36979), S_1	25	124	97	32	12	5	1	2	0	2	0	0	0	1	0	1	0	0	1	0	1	304
B_1 [F_1 (Fairfax × 36979) × 36979]	158	121	60	21	6	1	2	0	1	0	0	0	1	0	0	0	0	0	1	0	0	372
F_1 (Fairfax × 36979)	3	19	26	30	19	24	18	5	9	4	4	3	5	5	1	3	1	3	1	1	1	185
F_1 (Fairfax × 361477)	0	7	32	58	27	24	14	10	11	9	9	5	5	7	5	8	1	4	1	1	2	240
F_1 (Fairfax × 37501)	3	17	40	35	17	19	9	2	7	8	1	13	2	9	1	4	2	3	4	1	1	198
Subtotal	6	43	98	123	63	67	41	17	27	21	14	21	12	21	7	15	4	10	6	3	4	623
Subtotal grouped [1]		49	98	123	63	67	41	17	27		35		33		28		19				23	623
F_2 (Fairfax × 36979)	134	151	45	7	7	5	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	352
F_2 (Fairfax × 36979)	79	100	59	19	8	5	0	2	0	2	1	1	0	0	1	0	0	0	0	0	0	277
F_2 (Fairfax × 36979)	126	145	52	14	5	6	2	1	0	1	0	0	0	0	0	0	0	1	0	0	0	353
F_2 (Fairfax × 36979)	66	152	102	25	11	9	1	2	1	1	1	1	1	0	1	1	0	0	0	0	0	375
Subtotal	405	548	258	65	31	25	4	6	1	4	2	2	1	1	2	1	0	1	0	0	0	1,357
Subtotal grouped [1]	405	548	258	65	31	25												25				1,357
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	316	85	8	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	413
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	204	164	30	3	0	0	2	0	1	0	0	0	1	0	0	0	0	0	0	0	0	405
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	291	94	14	9	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	410
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	226	159	22	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	411
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	232	151	16	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	402
Subtotal	1,269	653	90	19	2	0	4	1	1	0	0	0	1	0	0	0	0	0	0	1	0	2,041
Subtotal grouped [1]	1,269	653	90																	29		2,041
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	58	189	104	26	8	4	3	2	0	0	0	0	0	0	0	0	1	1	0	0	0	396
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	43	164	106	30	13	1	3	2	1	0	0	0	0	0	0	0	0	0	0	0	0	363
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	56	156	94	23	12	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	346
Subtotal	157	509	304	79	33	9	6	4	2	0	0	0	0	0	0	0	1	1	0	0	0	1,105
Subtotal grouped [1]	157	509	304	79	33													23				1,105
B_1 [Fairfax × F_1 (Fairfax × 36979)]	2	12	45	41	18	43	14	12	14	4	5	8	6	4	3	0	3	0	0	0	0	234
B_1 [Fairfax × F_1 (Fairfax × 36979)]	11	22	50	53	30	45	6	14	12	9	7	4	4	1	4	0	2	0	1	2	1	278
B_1 [Fairfax × F_1 (Fairfax × 36979)]	4	10	67	39	20	22	11	6	7	8	5	3	4	7	4	6	2	4	2	5	2	238
B_1 [Fairfax × F_1 (Fairfax × 36979)]	17	48	56	37	18	32	14	8	5	10	10	6	4	2	5	0	1	2	0	0	0	275
B_1 [Fairfax × F_1 (Fairfax × 36979)]	10	58	40	26	28	8	7	9	6	9	7	7	3	7	5	4	1	0	0	0	2	237
Subtotal	44	150	258	196	114	150	52	49	44	40	34	28	21	21	21	10	9	6	3	7	5	1,262
Subtotal grouped [1]	44	150	258	196	114	150	52	49	44	40	34	28		42		31					30	1,262
× *F. ananassa* (Fairfax), S_1	3	7	12	20	21	17	6	8	11	7	3	1	6	2	3	1	0	2	1	1	0	132
Theoretical	37.9	39.5	21.6	10.2	5.2	5.0	2.1	1.6	1.6	1.4	1.0	1.0	0.8	0.8	0.6	0.5	0.3	0.3	0.2	0.2	0.2	132
Theoretical grouped	37.9	39.5	21.6	10.2	5.2	5.0				6.7											5.9	132
Grand total	2,067	2,155	1,177	555	282	274	116	87	87	74	53	52	42	46	33	28	14	20	12	12	10	7,196

[1] Each subtotal grouped includes the totals in the preceding line that are not included in the immediately preceding subtotal grouped.

For example, to test whether differences existed between populations as regards time of germination, the theoretical for the populations (in this case selfed Fairfax, table 1) having the smallest total number was calculated and the data were grouped accordingly. Grouping was also necessary for testing whether the differences noted within populations as to time of germination were statistically significant. The groupings of table 1 account for the fact that the total degrees of freedom listed for time of germination (table 2) are the sum of the degrees of freedom for grouped data (105), for between means of populations (49), and for periods (7).

TABLE 2.—χ^2 *values for testing the statistical significance of the differences noted in table 1 between populations as regards the ratio between germinated and nongerminated seeds and the time of germination*

Variation due to—	Degrees of freedom for grouped data	Degrees of freedom	χ^2
Total		482	
Populations		22	
Periods		20	
Grouping on the basis of the theoretical number of seeds in each category		183	
Ratio between germinated and nongerminated seeds		22	
Between means of populations		7	1,759.333
Within populations		15	146.453
F_1 (Fairfax×36979)		2	15.563
F_2 (Fairfax×36979)		3	80.422
F_1 (Fairfax×36979)×F_1 (Dorsett×37501)		4	[1] 5.131
F_1 (Fairfax×36979)×F_1 (Gem×361477)		2	26.321
B_1 [Fairfax×F_1 (Fairfax×36979)]		4	19.016
Time of germination		[2] 161	
Periods		7	
Between means of populations		49	4,715.474
Within populations		118	409.637
F_1 (Fairfax×36979)	14	24	38.551
F_2 (Fairfax×36979)	21	18	76.702
F_1 (Fairfax×36979)×F_1 (Dorsett×37501)	28	12	101.913
F_1 (Fairfax×36979)×F_1 (Gem×361477)	14	10	[1] 7.934
B_1 [Fairfax×F_1 (Fairfax×36979)]	28	54	184.537

[1] Odds against the deviations noted being due to chance are less than 19 to 1 ($P>0.05$).
[2] Sum of degrees of freedom for periods, for between means of populations, and for grouped subtotals.

The percentages of the seeds that germinated follow:

Population:	*Germination (percent)*
D. C. [F_1 (Fairfax×36979)×F_1 (Dorsett×37501)]	96.0
B_1 [F_1 (Fairfax×36979)×36979]	87.5
D. C. [F_1 (Fairfax×36979)×F_1 (Gem×361477)]	86.7
F_2 (Fairfax×36979)	79.8
Fragaria ovalis (36979), S_1	71.5
B_1 [Fairfax×F_1 (Fairfax×36979)]	59.4
F_1 (Fairfax×36979)	48.9
× *F. ananassa* (Fairfax), S_1	31.1

From these percentages and the calculations of table 2, it is apparent that the populations differ in the percentage of seeds that germinated during the 140-day period. Since the parents were not homozygous, and hence the F_1 populations might differ, it seemed probable that differences in percentage of germination might occur within populations. Such was found to be the case. However, the variation was not nearly so great within populations as between populations. Thus

it appears that variation due to segregation of genes within the parents was small as compared with that due to genetic differences between parents.

The F_1 (Fairfax × 36979) was used as the maternal parent in producing the seeds for the two double crosses, the backcross to 36979, and the F_2 populations; Fairfax was used as the maternal parent in producing the seed for the backcross to Fairfax, the F_1 progenies, and self-pollinated Fairfax; and of necessity, *F. ovalis* 36979 was used as the maternal parent in producing the seeds for self-pollinated 36979. When the F_1 hybrid was used as the female parent the percentage of the seeds that germinated was higher than when some other female parent was used. This would seem to indicate that the genotype of the maternal parent played a dominant role in determining the percentage of germination. If such actually were the case, there should be a difference between reciprocal crosses. The data on reciprocal crosses, given in table 3, show that when the F_1 hybrid was used as the female parent the percentage of germination in 140 days was 95.4, whereas when Fairfax was used as the female parent the percentage was 68.0. Of the seeds resulting from self-pollinating Fairfax, 62 percent germinated. It is evident that the genotype of the maternal parent played a dominant part in determining the percentage of germination.

With this fact established, the row totals for table 1 and the percentages for the different variates, previously listed, may be interpreted. The average percentage of germination for those populations having the F_1 hybrid as the female parent was 87.5; for those having Fairfax as the female parent, 54.2; for self-pollinated collection 36979, 71.5; and for self-pollinated Fairfax, 31.1. It is evident that heterosis exists as regards the effect of the maternal parent upon germination.

TABLE 3.–*Frequency distributions of germinating strawberry seeds approximately 2½ years old from reciprocal crosses and self-pollinated Fairfax*

Population	Seeds germinating after being in the germinator for the indicated number of days															
	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
F_1 (Fairfax × 36979) × Fairfax	123	174	64	25	10	11	15	16	1	2	3	2	0	3	10	3
Fairfax × F_1 (Fairfax × 36979)	3	45	56	17	10	9	14	21	12	6	6	6	2	10	38	17
Fairfax (S_1)	0	33	41	14	5	6	12	24	5	2	5	5	2	6	30	18
Total	126	252	161	56	25	26	41	61	18	10	14	13	4	19	78	38

Population	Seeds germinating after being in the germinator for the indicated number of days												Total	Germination	Mean time for germination
	85	90	95	100	105	110	115	120	125	130	135	140			
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*Pct.*	*Days*
F_1 (Fairfax × 36979) × Fairfax	3	1	4	0	1	2	2	2	0	0	0	0	477	95.4	18
Fairfax × F_1 (Fairfax × 36979)	20	9	7	9	4	5	9	3	1	1	0	0	340	68.0	49
Fairfax (S_1)	21	13	16	4	5	15	11	4	10	2	1	0	310	62.0	60
Total	44	23	27	13	10	22	22	9	11	3	1	0	1,127	------	------

That the genotype of the maternal parent is not the sole factor controlling germination is shown by the fact that there were differences, not accountable for by chance variation, between germination percentages of the populations having the F_1 hybrid as the maternal parent. The range was from 79.8 to 96.0 percent. Moreover, the percentages of germination for the backcross to Fairfax and the F_1 were greater than that for self-pollinated Fairfax; yet Fairfax was the maternal parent of all of them. These data as a whole show that the genotype of those parts of the seed resulting from fertilization plays a part in determining the percentage of germination as well as does the genotype of the maternal parent.

The data for time of germination are given in table 1, and the heterogeneity χ^2's for testing the significance of differences in table 2. There were marked differences between the means of hybrid populations and parents as to time of germination. This is also true, with the exception of the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Gem $\times$ 361477)], of the variates within hybrid populations. However, as noted in the case of percentages of germination, these differences are small as compared with the differences between means of hybrid populations and parents.

To facilitate the study of the data on time of germination the percentages of germinated seeds were calculated for the grouped subtotals (table 4). Without exception the populations having the F_1 hybrid as the maternal parent were the earliest to germinate and those having Fairfax as the maternal parent were the latest. These are the findings that would be expected if the maternal parent has a dominant influence on time of germination. To determine whether such was actually the case, reciprocal crosses involving the backcross to Fairfax were tested for time of germination (table 3). The data show that the backcross to Fairfax germinated considerably earlier when the F_1 hybrid was used as the female parent than when Fairfax was used as the female parent. Thus, from the data of tables 1 to 4, it may be concluded that the maternal parent had a predominant effect upon time of germination and that the F_1 hybrid exhibits decided heterosis in this respect.

TABLE 4.—*The percentage of the viable seeds that germinated during the indicated number of days after the seed had been put in the germinators (data from table 1)*

Population	Seeds germinating after being in germinator for indicated number of days								Mean for germination period
	40	45	50	55	60	65	70–85	90–140	
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Days*
D. C. [F_1(Fairfax×36979)×F_1(Dorsett×37501)]	62.2	32.0	4.4	0.9	0.1	0.0	0.3	0.1	42
B_1 [F_1 (Fairfax×36979)×36979]	42.5	32.5	16.1	5.7	1.6	.3	.8	.5	45
F_2 (Fairfax×36979)	29.8	40.4	19.0	4.8	2.3	1.8	1.1	.8	46
D. C. [F_1(Fairfax×36979)×F_1(Gem×361477)]	14.2	46.1	27.5	7.1	3.0	.8	1.1	.2	47
Fragaria ovalis (36979), S_1	8.2	40.8	31.9	10.5	4.0	1.6	1.7	1.3	50
B_1 [Fairfax×F_1 (Fairfax×36979)]	3.5	11.9	20.4	15.5	9.0	11.9	14.7	13.1	64
F_1 (Fairfax×36979), F_1 (Fairfax×361477), and F_1 (Fairfax×37501)	1.0	6.9	15.7	19.7	10.1	10.8	17.0	18.8	69
×*F. ananassa* (Fairfax), S_1	2.3	5.3	9.1	15.2	15.9	12.9	24.2	15.1	69

That the genotype of those parts of the seed resulting from fertilization also had a decided effect upon time of germination is shown by the differences in time of germination between the populations having the F_1 hybrid as the maternal parent and again by the differences in time of germination between the populations having Fairfax as the maternal parent (table 4). The 2½-year-old seed started germination 35 days earlier than the 1-year-old seed. For the 2½-year-old seed the percentage of germination for the backcross to Fairfax was 8.6 percent higher than that of the 1-year-old seed, and for self-pollinated Fairfax, 30.9 percent higher.

It will be remembered that there were differences within populations as to time of germination, which would be due to genetic disparity within species. However, the variation due to differences in genotypes of the species was considerably greater than that due to genetic differences within species. The genetic differences within species probably were due to differences between varieties or collections of the species and to heterozygosity of these varieties or collections.

The foregoing studies on germination of strawberry seeds have a direct bearing upon a strawberry-breeding program. Reciprocal crosses do not necessarily behave alike and since the maternal parent has a preponderance of influence upon the percentage and time of germination, the breeder may well afford to determine which way his crosses should be made so as to give the greatest number of seedlings in the shortest time. It seems probable that in most crosses involving × *Fragaria ananassa* and *F. ovalis* the F_1 hybrid when used as the female parent will show heterosis as regards the maternal effect upon germination. So, other things being equal, time and labor may be saved by using the F_1 hybrids as the female parent in obtaining seeds for the production of the backcross generations.

Probably the most important result of these studies is the insight they furnish as to the possibilities for selection. As many as 96 percent of the seeds of some of the material germinated, and some were considerably earlier in germinating than others. This degree of genetic disparity in the material indicates that by selection in segregating hybrid populations the breeder may be able to build up stocks that will possess a high percentage of germination and will germinate quickly. This conclusion is in accord with the findings of Darrow et al. (*3*) and Henry (*8*). Their investigations show that both percentage and time of germination may vary according to species and variety.

FIELD STUDIES

As previously stated, the primary objective of the breeding program was to recombine the winter hardiness of the native Rocky Mountain strawberry with the fruit size of the cultivated varieties. Success in attaining this objective is affected by (1) the dominance and heterosis relations of the characters, (2) the frequency of occurrence of the desirable characters in the hybrid populations in which there is a possibility of these characters being recombined, (3) whether the genes differentiating the characters under consideration are independently inherited or are linked, (4) whether pleiotropy (multiple effects of a gene) is occurring as regards some or all of the genes differentiating certain characters, and (5) the effect of the environment on the rela-

tion between the characters. Ten characters were selected for study. The data in respect to these are divided into three parts. The first treats of dominance and heterosis, the second of the frequency of occurrence of the desirable character in the populations in which there is a possibility of these characters being recombined, and the third of the independence of the characters, of pleiotropy, and of the influence of the environment upon the relation between the characters.

Dominance and Heterosis

Probably one of the best ways of determining whether dominance and heterosis exist is to compare the means of the populations and parents. Before proceeding further, it is desirable to have a clear conception of dominance and heterosis. Even though, from the standpoint of physiological genetics, dominance and heterosis are probably fundamentally the same, it is convenient in these studies to use both terms. Complete dominance is applied to those cases in which the phenotypic expression in the F_1 hybrid is that of one or the other parent. The term "partial dominance" is used when the phenotypic expression in the F_1 hybrid lies between those of the parents but is anything other than exactly intermediate arithmetically. Those cases are considered as exhibiting heterosis in which the phenotypic expression of a character in the F_1 hybrid is greater than the magnitude of such expression in both parents. It should be pointed out that even though means of the F_1 compared with those for the parents provide the most information concerning dominance and heterosis, comparisons involving the means of other populations are valuable in furnishing supplementary evidence.

The means and their standard errors for the 10 characters studied are given in table 5. The grades for winter injury listed in the second column show that in some cases the winter injury of the F_1 hybrid and of the double cross [F_1 (Fairfax$\times$36979)$\times$$F_1$ (Gem$\times$361477)] is no greater than that of the respective *Fragaria ovalis* parents and in the remaining cases it is almost as low. Hence, for all practical purposes the winter hardiness of the *F. ovalis* collections is almost if not completely dominant. Differences in degree of winter hardiness exist within both *F. ovalis* and $\times$ *F. ananassa*, but these differences are minor as compared with the differences between the two.

All but two of the plants of Fairfax and all the plants of Dorsett winter-killed. Hence, for these two varieties there were no strictly comparable data for the characters other than winter injury and number of runners. Consequently, with the exception of the two characters mentioned, the means of Fairfax self-pollinated and Gem asexually propagated are used in the comparisons involving $\times$ *Fragaria ananassa*.

The data of table 5 show that the fruits of selfed 36979 averaged somewhat smaller than those of asexually propagated 36979, that the means of the F_2 populations were consistently lower than those of the F_1 populations, and that means of the double crosses were consistently greater than those of the F_2 populations. However, in this latter comparison the differences were not marked. Hence, under the environmental conditions encountered at Cheyenne large size of fruit is partially dominant.

TABLE 5.—*Means and standard errors of the populations*

(The larger the number the greater the degree of winter injury; the larger the size of the fruit; the greater the number of runners; the greater the sweetness of the fruit; the more desirable the flavor; and the firmer the flesh]

Population	Grade for winter injury	Grade for size of fruit	Plant height	Grade for number of runners	Period from May 1 to first bloom	Period from first bloom to first fruit ripe	Period from May 1 to first fruit ripe	Grade for sweetness of fruit	Grade for flavor of fruit	Grade for firmness of fruit
			Centimeters		*Days*	*Days*	*Days*			
Fragaria ovalis:										
37501 (asexual)	1.690±0.282	1.360±0.065	12.9±0.433	3.490±0.122	14.8±1.083	41.1±0.994	55.9±0.849	2.240±0.064	2.450±0.099	2.740±0.052
361477 (asexual)	.940± .133	1.044± .067	11.6± .400	3.380± .143	15.0± .365	48.4±1.473	63.4±1.481	1.922± .106	1.933± .091	2.967± .033
36979 (asexual)	1.000± .170	.810± .057	11.3± .597	3.050± .114	14.2± .696	46.0± .943	60.2± .611	2.630± .087	2.700± .082	2.900± .037
36979 (S_1)	1.060± .124	.660± .037	8.8± .554	2.640± .103	14.5± .477	49.1± .605	63.6± .819	2.720± .074	2.700± .082	2.890± .048
Hybrid populations:										
B_1 [F_1 (Fairfax × 36979) × 36979]	1.070± .141	1.350± .040	16.3± .633	3.260± .070	14.3± .517	44.6± .702	58.9± .657	2.380± .090	2.460± .067	3.000± .068
F_1 (Fairfax × 36979)	1.320± .146	1.870± .073	17.7± .668	2.840± .085	16.3± .260	40.8± .389	57.1± .407	2.690± .105	2.670± .100	3.130± .033
F_1 (Fairfax × 361477)	1.030± .086	1.690± .077	19.1± .526	2.830± .078	17.4± .427	40.8± .573	58.2± .680	2.280± .063	2.430± .067	3.200± .037
F_1 (Fairfax × 37501)	1.300± .058	2.083± .065	18.7± .667	2.717± .095	18.0± .816	38.3± .615	56.3± .558	2.450± .123	2.550± .096	3.233± .099
F_2 (Fairfax × 36979)	2.310± .145	1.390± .066	13.4± .427	2.930± .040	23.2± .573	39.9± .605	63.1± .567	2.580± .076	2.630± .073	3.300± .037
F_2 (Fairfax × 36979)	1.920± .167	1.540± .043	13.2± .554	3.110± .115	21.3± .731	41.4± .452	62.7± .396	2.780± .047	2.900± .045	3.240± .048
F_2 (Fairfax × 36979)	1.870± .141	1.490± .072	12.7± .473	3.010± .085	22.5± .687	40.2± .291	62.7± .746	2.810± .050	2.850± .052	3.250± .054
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	1.540± .109	1.790± .059	19.4± .653	3.520± .088	17.0± .422	39.7± .651	56.7± .396	2.520± .070	2.610± .095	3.160± .037
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	1.660± .119	1.880± .061	20.3± .633	3.490± .050	16.7± .396	39.3± .517	56.0± .394	2.330± .078	2.410± .102	3.110± .041
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	1.610± .157	1.780± .074	19.0± .394	3.400± .086	17.0± .365	39.9± .277	56.9± .348	2.380± .068	2.520± .101	3.160± .056
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	1.580± .137	1.780± .071	19.8± .611	3.560± .087	16.5± .500	40.7± .539	57.2± .593	2.510± .081	2.650± .056	3.190± .043
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	1.050± .079	1.690± .028	15.5± .401	3.200± .097	17.4± .670	39.8± .663	57.2± .490	2.240± .054	2.350± .065	3.030± .033
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	1.080± .131	1.770± .073	14.2± .442	3.230± .063	16.8± .442	40.6± .521	57.4± .542	2.410± .059	2.660± .062	2.960± .052
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	1.340± .176	1.850± .089	14.8± .573	3.180± .074	18.3± .473	39.8± .416	58.1± .586	2.460± .037	2.550± .048	3.070± .042
B_1 [Fairfax × F_1 (Fairfax × 36979)]	2.750± .132	2.140± .075	13.9± .674	2.510± .084	25.3± .895	37.2± .611	62.5± .601	2.870± .082	2.950± .085	3.210± .050
B_1 [Fairfax × F_1 (Fairfax × 36979)]	2.790± .195	2.120± .098	13.8± .359	2.470± .092	25.7± .578	35.9± .795	61.6± .702	2.910± .066	2.980± .066	3.260± .022
B_1 [Fairfax × F_1 (Fairfax × 36979)]	2.840± .224	2.150± .075	14.8± .593	2.450± .096	24.5±1.046	35.9± .767	60.4± .806	2.880± .071	2.920± .090	3.300± .033
× *F. ananassa:*										
Fairfax (S_1)	4.650± .169	2.100± .243	9.6± .748	1.167± .084	31.8±2.332	33.0±1.265	64.8±1.800	2.880± .073	2.920± .097	3.280± .058
Fairfax (asexual)	5.100± .165			1.471± .097						
Gem (asexual)	4.210± .122	2.900± .087	8.8± .512	1.330± .087	30.8±1.218	31.7± .651	62.5± .922	2.170± .054	2.290± .059	2.920± .039
Dorsett (asexual)	5.225± .111			1.025± .165						

From the data on plant height it can be seen that the backcross to *Fragaria ovalis*, the F_1 population, the F_2 population, both double crosses, and the backcross to Fairfax possessed greater height than did either Fairfax or 36979. Clearly plant height exhibits heterosis. The plants of the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)] averaged as tall as those of the F_1 hybrids. This fact substantiates the field observations that the plants of this double cross were as vigorous as those of the F_1 hybrids. In fact, the extreme vegetative vigor of the F_1 populations and this double cross was so noticeable that statistical substantiation of this conclusion was not necessary. The purpose of making the double crosses was to allow for segregation of the genes differentiating the interspecific characters, but yet to retain as much as possible of any heterosis the F_1 hybrid might possess. It seems that the vegetative vigor of the F_1 hybrid can be retained by certain double crosses involving $\times$ *F. ananassa* and *F. ovalis*.

Turning to number of runners, it is clear, from column 5 of table 5, that the means of the F_1 hybrids approach more closely the means of the collection of *Fragaria ovalis* than they do those of the varieties of $\times$ *F. ananassa*. Hence, the tendency to produce a large number of runners is at least partially dominant to the tendency to produce a small number of runners.

For days from May 1 to first bloom the means of the F_1 hybrid approach rather closely those of the *Fragaria ovalis* collections, indicating a fairly high degree of partial dominance of early blooming. By the same criterion, the short period from first bloom to first fruit ripe is partially dominant, but the degree of dominance is slight. The addition of these two periods gives the days from May 1 to first fruit ripe, for which the F_1 hybrid exhibits a slight degree of heterosis. These conclusions regarding dominance and heterosis are substantiated by the fact that the means for the two double crosses are not materially different from those of their respective F_1 hybrids. Again, the desirable characteristics of the F_1 hybrids have been maintained in the double crosses. In days from May 1 to first bloom the collections of *F. ovalis* are earlier than the varieties of $\times$ *F. ananassa*, whereas in days from first bloom to first fruit ripe $\times$ *F. ananassa* has the shorter period. Consequently, the differences between the two species are slight as regards days from May 1 to first fruit ripe. This would indicate that the plant breeder may have considerable difficulty in combining these two phases of earliness.

For sweetness and flavor of fruit the data show the differences within species to be greater than the differences between species. For this reason the data are not conclusive in respect either to dominance or heterosis. However, one may conclude that in crosses involving *Fragaria ovalis* and $\times$ *F. ananassa* it should not be difficult to select plants possessing sweet fruits of high flavor.

Firmness of fruit presented no serious problem in these crosses. In general the fruits of the native Rocky Mountain strawberries used in the crosses were not as firm as those of the cultivated varieties. The means of the F_1 hybrid approached rather closely the mean of self-pollinated Fairfax, indicating at least partial dominance of firm fruit. However, as the differences between species were not very great this conclusion is of no great significance and probably not very well established.

Frequency of Occurrence of Characters

The number of individuals in each class and the percentage of the individuals in the more desirable class, when the characters are divided into two classes, are listed in table 6. Chi square was used in testing the significance of differences noted (see Snedecor *16, pp. 19, 168–171*).

Plants shown in table 6 as having a grade for winter injury equal to or less than 0.5 or 1.0 are extremely winter hardy. In fact, a high degree of winter hardiness as compared with commercial varieties is possessed by any selection that averages 2.5 or less. Of the varieties of × *Fragaria ananassa*, only one plant (Gem asexually propagated) received a grade as low as 1.0. Of the segregating populations, the backcross to 36979 had the greatest proportion of its plants in the more desirable class for winter hardiness. However, the fruits of these plants were too small to be commercially acceptable. Next in order were the F_1 hybrids and the two double crosses. The F_1 hybrids and the double cross [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)] had somewhat more than 50 percent of their plants in the desirable class, whereas 40 percent of the plants of the other double cross fell in this class. As was to be expected of the hybrid populations, the backcross to Fairfax had the smallest percentage in the more desirable class. Probably the fact of greatest importance to a plant-breeding program was that plants possessing degrees of winter hardiness from 0.5 to 1.0 occurred frequently enough to allow selection for other characters.

The data on size of fruit (table 6) are of special interest because of the light they shed on the breeding behavior of this character. The greatest frequency of occurrence of plants having fruits of a size grade of 3.0 or greater was found in the F_1 population of Fairfax × 37501 and the backcross to Fairfax. Since Fairfax is the large-fruited parent, it would be expected that the highest percentage of large-fruited plants would be found in the backcross to Fairfax; but such was not the case. Why this particular F_1 hybrid should have had such a high proportion of large-fruited plants is not clear. A logical supposition is that *F. ovalis* may carry some genes for large size of fruit. However, in view of the work reported by Hildreth and Powers (*9*), this does not seem probable. Among the plants of 42,000 collections involving 1,100 locations, they found that none possessed fruits that would approach a size grade of 2.5. So large a number of collections from so many locations should form an adequate sample for testing what would be expected in the way of segregation within *F. ovalis*. Therefore, it seems probable that *F. ovalis* does not carry any genes for large size of fruit. Such being the case, it seems likely that the explanation is to be found in dominance relations. It will be remembered that large size of fruit was at least partially dominant. If in *F. ovalis* there were some genes that were completely or almost completely recessive to the genes of Fairfax for production of large fruit, then, by segregation within the *F. ovalis* parent, the large-fruited segregates obtained among the F_1 populations would be expected even though, comparatively speaking, no large-fruited *F. ovalis* plants have ever been found.

The question arises as to whether these large-fruited F_1 plants are true F_1 plants or false hybrids. The fact that they possess extreme winter hardiness and exhibit other characteristics of the *Fragaria*

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TABLE 6.—*Classification of populations and percentage of individuals in the more desirable class when the characters are divided into 2 classes*

[Observational grades were used when the units of measurement are not stated]

Population	Winter injury			Size of fruit			Plant height in centimeters			Number of runners			Days from May 1 to first bloom			Days from first bloom to first fruit ripe			Days from May 1 to first fruit ripe			Sweetness of fruit			Flavor of fruit			Firmness of fruit		
	0.5–1.0	1.5–5.5	0.5–1.0	0.5–2.5	3.0–5.5	3.0–5.5	1–17	18–31	18–31	0.5–3.0	3.5–5.5	3.5–5.5	5–14	17–62	5–14	18–33	36–69	18–33	50–53	56–89	50–53	0.5–3.0	3.5–5.5	3.5–5.5	0.5–3.0	3.5–5.5	3.5–5.5	0.5–3.5	4.0–5.5	4.0–5.5
Fragaria oralis:	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*	*No.*	*No.*	*Pct.*
37501 (asexual)	34	44	44	65	0	0	78	0	0	22	56	72	58	17	77	1	64	2	32	33	49	65	0	0	61	4	6	65	0	0
361477 (asexual)	71	22	76	27	0	0	93	0	0	44	49	53	68	25	73	0	31	0	2	29	6	27	0	0	26	1	4	27	0	0
36979 (asexual)	77	19	80	72	0	0	96	0	0	65	31	32	81	14	85	0	72	0	8	64	11	65	7	10	65	7	10	72	0	0
36979 (S_1)	81	26	76	61	0	0	104	0	0	91	18	17	81	21	79	0	64	0	3	61	5	57	4	7	58	3	5	61	0	0
Hybrid populations:																														
B_1 [F_1 (Fairfax × 36979) × 36979]	84	31	73	106	0	0	74	39	35	51	65	56	90	23	80	0	106	0	23	83	22	104	2	2	94	12	11	101	5	5
F_1 (Fairfax × 36979)	68	40	63	91	5	5	50	53	51	82	26	24	41	60	41	0	96	0	26	70	27	75	21	22	74	22	23	91	5	5
F_1 (Fairfax × 361477)	80	37	68	110	3	3	33	84	72	99	18	15	42	74	36	2	111	2	15	98	13	110	3	3	101	12	11	104	9	8
F_1 (Fairfax × 37501)	35	32	52	55	12	18	22	45	67	52	15	22	23	44	34	11	56	16	25	42	37	60	7	10	57	10	15	55	12	18
F_2 (Fairfax × 36979)	110	234	32	258	3	1	301	36	11	226	118	34	35	264	12	33	229	13	11	252	4	229	32	12	201	60	23	230	30	12
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	184	275	40	409	27	6	122	337	73	151	308	67	221	226	49	34	402	8	159	277	36	402	34	8	355	81	19	400	36	8
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	226	122	65	319	18	5	272	75	22	185	163	47	134	210	39	24	313	7	98	239	29	317	20	6	289	48	14	327	10	3
B_1 [Fairfax × F_1 (Fairfax × 36979)] × *F. ananassa:*	39	265	13	189	41	18	240	53	18	288	16	5	16	240	6	65	166	28	30	201	13	175	55	24	155	75	33	210	20	9
Fairfax (S_1)	0	58	0	10	3	23	34	1	3	58	0	0	0	18	0	5	8	38	1	12	8	11	2	15	10	3	23	13	0	0
Fairfax (asexual)	0	15	0	2	0	0	2	0	0	15	0	0	0	2	0	2	0	100	0	2	0	2	0	0	2	0	0	2	0	0
Gem (asexual)	1	109	1	21	43	67	87	0	0	112	0	0	68	0	50	15	77	0	65	0	63	1	2	62	2	3	62	2	3	
Dorsett (asexual)	0	21	0	0	0	0	1	0	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

ovalis parent, such as the ability to produce large numbers of runners, would rule out the probability of their being false hybrids. Further support of this conclusion is the fact that the selfed progeny of Fairfax had no plants that exhibited a high degree of winter hardiness.

The percentages for the different populations of the plants that would be expected to have both extreme winter hardiness and large size of fruits follow:

Population:	*Percent*
B_1 [F_1 (Fairfax × 36979) × *Fragaria ovalis* 36979]	0. 00
F_1 (Fairfax × *F. ovalis* 36979)	3. 15
F_1 (Fairfax × *F. ovalis* 361477)	2. 04
F_1 (Fairfax × *F. ovalis* 37501)	9. 36
F_2 (Fairfax × 36979)	. 32
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	2. 40
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	3. 25
B_1 [Fairfax × F_1 (Fairfax × 36979)]	2. 34

The most striking feature of these data is the theoretical possibility for selection among the F_1 plants of Fairfax × 37501. One other fact, which the data do not bring out, is that the fruits of the backcross to Fairfax falling in the desirable size group will average considerably larger than those for any of the other populations.

The greatest possibilities for selection of the desirable type for plant height, other considerations being equal, are offered by the F_1 hybrids and the double cross [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)] (table 6). This was to be expected because of the greater heterosis shown by these populations. It is apparent that the possibilities are good for obtaining vigorous selections.

Among the populations having some large-fruited segregates, the chances of selecting plants producing a large number of runners are greatest for the double cross [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]. The other double cross ranks second in this respect. Again, the occurrence of the desirable type is sufficiently frequent to provide good opportunities for selection. It should be pointed out that under certain cultural practices the plants producing few runners are desirable. However, this is not true in the central Great Plains and Rocky Mountain regions.

The frequencies of occurrence of the classes for the different phases of earliness will be considered together. Among the segregating populations having some plants with large fruits, the F_1 plants and the double crosses offered the greatest possibilities for selecting those having a short period from May 1 to first bloom. This was not true for days from first bloom to first fruit ripe, as the backcross to Fairfax had the largest percentage of plants in the desirable class. For days from May 1 to first fruit ripe the double crosses and the two F_1 populations involving 36979 and 37501 had the largest percentage of plants in the desirable class.

In considering sweetness, flavor, and firmness of fruit it should be pointed out that the two species did not differ greatly except possibly in respect to firmness (table 5). The parents from both species, with

the possible exception of Gem and collection 361477, possessed high-quality fruit. The more desirable classes for which the percentages are given represent the plants having extremely high-quality fruits; hence many plants in the other class had fruits of very good quality. In fact, quality in these hybrids is a minor problem as the majority of segregates had fruits of good quality. Whenever Gem, 361477, or both entered into a cross the proportion of plants having extremely high quality was reduced (table 6).

Frequency of Recombination of Desired Characters

If the relation of two characters is such that they are independent in the segregating populations, then the frequency of the occurrence of individuals recombining the two characters can be predicted. For example, the proportion of the plants of the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)] having a grade of 1.0 or less for winter injury was 0.400871 and the proportion of the plants of the same cross having a plant height falling within 18 to 31 cm. was 0.734205. The expected proportion of the total number of plants that recombined both characters is, then, 0.400871 $\times$ 0.734205, or 0.294321; and the expected proportion that possessed the alternative characters is 0.705679. Chi square was employed to test whether the differences between the expected numbers and those obtained could be accounted for by chance.

In obtaining these χ^2 values, when consistent with the biological considerations of table 6, the data were grouped so that the expected number for either of the two classes was not less than five. When this was not advisable the difficulties arising from small numbers (*6, p. 96*) were taken into consideration. The χ^2 values are listed for each category of table 7. Both are necessary for a complete analysis of the data. One of the advantages of the second χ^2 is that the expected numbers are large.

If the differences between the obtained and expected numbers cannot be attributed to chance variation, then linkage, pleiotropy, environment, or a combination of these may be responsible for the discrepancies noted. By calculating the χ^2 values for all the populations of table 6, information may be obtained as to which of these factors are operating to produce the discrepancies noted. All these χ^2 values were calculated, but only those of the five populations segregating for the genes differentiating the two species are given in table 7. The data of this table will be discussed on the basis of the row designations. For example, when discussing the χ^2 values for the row designated winter injury the χ^2 values for all the other characters and winter injury are discussed; whereas for size of fruit the χ^2 value for this character and winter injury are not included, as it would have been discussed previously under the row designated winter injury.

TABLE 7.—χ^2 *values for testing independence of the different characters calculated from the populations segregating for the genes differentiating the species*

[The χ^2 listed first under each column and each row is the sum of the χ^2's for the individual generations and has 5 degrees of freedom; whereas the χ^2 listed second under each column and each row is calculated from the totals of the expected and the obtained for all the populations and has but 1 degree of freedom]

Character	Size of fruit	Plant height	Number of runners	Days from May 1 to first bloom	Days from first bloom to first fruit ripe	Days from May 1 to first fruit ripe	Sweetness of fruit	Flavor of fruit	Firmness of fruit
Winter injury	6.787	32.528*	6.121	6.902	1.858	7.372	4.438	3.914	5.585
	.393	11.936*	2.714	3.070	.201	3.413	2.516	1.915	3.637
Size of fruit	-----	5.005	2.216	19.139*	30.908*	244.281*	13.416*	13.090*	1.123
	-----	1.558	.181	13.618*	18.656*	194.811*	8.105*	8.722*	.426
Plant height	-----	-----	15.730*	11.691*	1.939	5.727	4.974	2.268	3.281
	-----	-----	6.559*	3.012	.001	2.270	.246	.018	1.718
Number of runners	-----	-----	-----	16.805*	1.857	9.474	2.013	.895	2.588
	-----	-----	-----	8.557*	1.105	3.587	.534	.288	.318
Days from May 1 to first bloom	-----	-----	-----	-----	68.877*	93.669*	2.555	2.305	1.510
	-----	-----	-----	-----	66.294*	42.556*	.344	.990	.402
Days from first bloom to first fruit ripe	-----	-----	-----	-----	-----	36.510*	4.582	2.915	.553
	-----	-----	-----	-----	-----	28.535*	2.235	1.615	.020
Days from May 1 to first fruit ripe	-----	-----	-----	-----	-----	-----	3.603	5.101	12.231*
	-----	-----	-----	-----	-----	-----	.909	1.365	5.792*
Sweetness of fruit	-----	-----	-----	-----	-----	-----	-----	295.546*	.484
	-----	-----	-----	-----	-----	-----	-----	224.200*	.138
Flavor of fruit	-----	-----	-----	-----	-----	-----	-----	-----	1.142
	-----	-----	-----	-----	-----	-----	-----	-----	.610

*$P<0.05$, indicating that the deviations noted cannot be attributed to chance.

From table 7 it can be seen that with the exception of plant height none of the χ^2 values for winter injury and the other characters are of sufficient magnitude to warrant the conclusion that the genes differentiating them are not independently inherited. It remains to be seen whether linkage, pleiotropy, environment, or a combination of these factors is responsible for the association noted between winter injury and plant height. Information pertaining to this phase of the problem may be obtained from table 8. The statistically significant χ^2 values are for those populations in which the proportion of non-winter-hardy plants would be expected to be greatest; namely, Gem, selfed Fairfax, the F_2 populations, and the backcross to Fairfax. The discrepancies noted for asexually propagated Gem cannot be attributed to linkage or pleiotropy. However, the facts observed are logically explained as due to environmental differences (winter injury to some plants was so severe as to reduce their height materially). If this deduction is correct, the obtained number for those populations having statistically significant χ^2's should in every instance exceed the expected. It will be noted that such is the case. Such a relation between the two characters does not affect the ability of a breeder to obtain the desired type by selection, because the environment affects the expected and not the obtained from which the breeder makes the selections. In other words, the environment has lowered the actual proportion of tall plants at the expense of the genetically potentially tall plants of the non-winter-hardy group. Since the expected number of plants combining hardiness and tallness of plant is calculated by multiplying the total proportion of tall plants expressed as a decimal fraction by the total proportion of winter-hardy plants expressed as a decimal fraction and this resultant fraction by the total number of individuals in the population, it is apparent that the expected has been lowered by this environmental action, whereas the obtained has

not. Therefore, as previously stated, the plant breeder has the same number and kind of individuals to select from as would have been the case if the environment had not had the effect noted.

TABLE 8.—χ^2 *values for testing whether the differences between the numbers obtained and those expected, if winter injury and plant height are independent, can be attributed to chance*

Population	Total plants	Plants combining winter hardiness and tallness		χ^2
		Obtained	Expected	
	Number	*Number*	*Number*	
Fragaria ovalis (37501), asexual	78	11	11.8	0.059
F. ovalis (361477), asexual	93	11	12.2	.139
F. ovalis (36979), asexual	96	14	14.4	.016
× *F. ananassa* (Gem), asexual	85	10	4.6	6.658*
Sum of χ^2's				6.872
χ^2 of totals	352	46	43.0	.233
F. ovalis (36979), S_1	104	8	6.1	.663
F_1 (Fairfax×36979)	103	38	33.4	.950
F_1 (Fairfax×37501)	67	22	23.5	.149
F_1 (Fairfax×361477)	117	59	57.4	.084
× *F. ananassa* (Fairfax), S_1	35	8	2.5	13.197*
Sum of χ^2's				15.043*
χ^2 of totals	426	135	122.9	1.688
B_1 [F_1 (Fairfax×36979)×36979]	113	29	28.5	.012
F_2 (Fairfax×36979)	337	21	11.5	8.097*
D. C. [F_1 (Fairfax×36979)×F_1 (Dorsett×37501)]	459	152	135.1	2.998
D. C. [F_1 (Fairfax×36979)×F_1 (Gem×361477)]	347	59	48.7	2.531
B_1 [Fairfax×F_1 (Fairfax×36979)]	293	18	6.8	18.889*
Sum of χ^2's				32.527*
χ^2 of totals	1,549	279	230.6	11.936

*$P<0.05$, indicating that the deviations noted cannot be attributed to chance.

Turning to size of fruit, it can be seen that all the χ^2 values other than those for plant height, runners, and firmness of fruit are statistically significant. Again, in every case, the number of individuals obtained for the class combining the desirable characters is greater than the number expected. The explanation for those characters that determine earliness of maturity seems to be that the environment has a similar effect upon them and size of fruit. It is evident that linkage of the genes that differentiate size of fruit with those that differentiate the earliness characters cannot be the explanation. On the other hand, the relation between size of fruit and sweetness and size of fruit and flavor seems to be due to linkage of the genes differentiating these characters, to pleiotropy, or to both.

The χ^2 values for plant height and number of runners are statistically significant. The sum of the χ^2's for plant height and days from May 1 to first bloom is also statistically significant, and the χ^2 for totals approaches statistical significance, if a P value of 0.05 or less is accepted as a criterion of statistical significance. Again, for the desirable combination of characters the obtained is greater than the expected. Since all of these characters are measures of plant vigor, it is not surprising that this is so. It would seem that pleiotropy surely is occurring, and linkage also would be expected to play some part. That none of the χ^2 values for the asexually propagated generations approached statistical significance supports these statements and

furnishes convincing evidence that the causes of these high χ^2 values are genetic. In any event, whether linkage or pleiotropy is responsible for the high χ^2 values, the task of the plant breeder is easier because of the greater proportion of plants combining the desirable characters from which selections can be made.

For number of runners, the only statistically significant χ^2 values are those listed opposite days from May 1 to first bloom. As before, the obtained class recombining the desirable characters is larger than the expected. The explanation is the same as that given in the preceding paragraph for plant height, runners, and days from May 1 to first bloom. In fact, this relation between runners and days from May 1 to first bloom was to be expected. It aids rather than hinders the breeding program.

Days from May 1 to first bloom, from first bloom to first fruit ripe, and from May 1 to first fruit ripe will be considered together. None of the χ^2 values for these characters and sweetness, flavor, or firmness of fruit is statistically significant, and hence will not be considered further. The χ^2 value for days from May 1 to first bloom and days from May 1 to first fruit ripe is statistically significant, as is also the χ^2 value for days from first bloom to first fruit ripe and days from May 1 to first fruit ripe. In all of these cases the obtained numbers of the desired class are greater than the expected numbers. None of the corresponding χ^2 values for the asexually propagated material were statistically significant. These facts show that the causes are genetic. These causes would be expected to be genetic since days from May 1 to first bloom plus days from first bloom to first fruit ripe gives days from May 1 to first fruit ripe. The χ^2 values for days from May 1 to first bloom and days from first bloom to first fruit ripe are statistically significant. From table 9, which gives all the χ^2 values for these two characters, it can be seen that none of the χ^2 values for the asexually propagated populations was statistically significant. For the first and only time the expected numbers were larger than those obtained for the desirable class. Since the genes differentiating the desirable characters entered the cross from different parents, it would seem that linkage, pleiotropy, or both are responsible for the high χ^2 values obtained. In any event the task of combining these two characters into a single individual to obtain a very early type with other desirable characters is much more difficult than if the genes differentiating the two characters had been independently inherited.

TABLE 9.—χ^2 *values for testing whether the differences between the numbers obtained and those expected, if days from May 1 to first bloom and days from first bloom to first fruit ripe are independent, can be attributed to chance*

Population	Total plants	Plants combining short period from May 1 to first bloom and short period from first bloom to first fruit ripe		χ^2
		Obtained	Expected	
	Number	*Number*	*Number*	
Fragaria ovalis (37501), asexual	65	3	4.6	0.624
F. ovalis (361477), asexual	31	6	5.8	.005
F. ovalis (36979), asexual	72	1	2.6	.984
× *F. ananassa* (Gem), asexual	65	35	36.5	.135
Sum of χ^2's				1.748
χ^2 of totals	233	45	49.5	.523
F. ovalis (36979), S_1	64	6	6.4	.022
F_1 (Fairfax × 36979)	96	0	5.3	5.581*
F_1 (Fairfax × 37501)	67	3	10.3	6.112*
F_1 (Fairfax × 361477)	113	0	8.3	8.990*
× *F. ananassa* (Fairfax), S_1	13	7	5.6	.656
Sum of χ^2's				21.364*
χ^2 of totals	353	16	35.9	12.198*
B_1 [F_1 (Fairfax × 36979) × 36979]	106	0	.8	.803
F_2 (Fairfax × 36979)	262	2	9.0	5.652*
D. C. [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]	436	19	61.3	33.972*
D. C. [F_1 (Fairfax × 36979) × F_1 (Gem × 361477)]	337	9	33.5	19.896*
B_1 [Fairfax × F_1 (Fairfax × 36979)]	231	0	8.2	8.555*
Sum of χ^2's				68.878*
χ^2 of totals	1,372	30	112.8	66.294*

* $P<0.05$, indicating that the deviations noted cannot be attributed to chance.

Sweetness, flavor, and firmness of fruit will be discussed together. None of the χ^2 values for firmness of fruit with either of the other two characters was statistically significant. Hence, the hypothesis of independence of this character of the other two was not disproved. The χ^2 values for sweetness and flavor of fruit were statistically highly significant. This was also true for the asexually propagated parents, showing that environment exerts some effect upon the χ^2 values. Again, the obtained numbers for the class combining the desirable characters were larger than the expected. How much, if any, of this relation can be attributed to linkage and pleiotropy cannot be ascertained from these data.

To summarize, it can be said that in only 1 of the possible 45 cases did linkage or pleiotropy interfere with the breeding program and in only 14 of the possible cases were the characters other than independent.

DISCUSSION AND CONCLUSIONS

PROMISING SELECTIONS

From the crosses between × *F. ananassa* and *F. ovalis*, 74 selections having large fruits and a high degree of winter hardiness have been chosen for increase and further testing. Already 3 of them have shown sufficient promise to warrant their release. Descriptions of these, the parents, and Gem are given in table 10.

During the past two winters all three selections have proved to be winter hardy. Their fruits are large and of very good quality. The fruits of the two F_1 hybrids are of good size but somewhat smaller than those of the cultivated varieties. This somewhat smaller size is more than compensated for, however, by their hardiness and earliness. The backcross to Fairfax has fruits as large as those of Dorsett and Fairfax but somewhat smaller than those of Gem. In addition, it is more prolific than either Dorsett or Fairfax. All three selections produced more runners than either Gem, Dorsett, or Fairfax, and the fruits of the two F_1 selections are very aromatic. The differences in leaf spotting between the selections and cultivated varieties were slight, the cultivated varieties being somewhat freer from leaf spots. Leaf spotting is not sufficiently serious in either to be of economic importance under the conditions existing at Cheyenne. The quality of these selections as regards sweetness and flavor is equal if not superior to that of Fairfax. The fruits of the F_1 selections are not quite so firm as those of Fairfax, and probably they would not be good shippers. However, the fruits of the backcross to Fairfax are firm and this selection should ship well.

TABLE 10.—*Description of the characters of 3 selections from crosses involving cultivated varieties and the native Rocky Mountain strawberry, 3 standard varieties, and 2 collections of Fragaria ovalis*

Selection, variety, or collection	Grade for winter injury	Grade for size of fruit	Height of plant	Grade for number of runners	Period from June 1 to first fruit ripe	Grade for sweetness of fruit	Grade for flavor of fruit	Grade for firmness of fruit	Grade for prolificacy of fruit	Grade for leaf spotting
			Centimeters		*Days*					
Fragaria ovalis:										
361261	0.5	1.0	14	3.0	22	2.0	2.5	2.5	2.0	2.0
36979	.5	1.0	14	3.5	23	2.7	2.7	2.9	2.0	2.0
Selections from hybrid populations:[1]										
F_1 (Dorsett × 361261)	1.0	2.8	17	3.5	10	3.5	3.5	3.5	4.1	1.0
F_1 (36979 × Fairfax)	1.0	3.0	19	3.2	21	3.8	3.7	3.5	2.5	1.0
Backcross [Fairfax × F_1 (Fairfax × 36979)] × *F. ananassa:*	1.2	3.5	22	3.0	34	3.5	3.5	4.5	3.5	1.0
Fairfax	4.8	3.5	12	1.5	36	3.5	3.5	4.0	2.5	.5
Dorsett	4.8	3.5	12	1.5	36	3.2	3.0	4.0	2.5	.5
Gem	4.0	4.0	10	2.0	34	2.1	2.5	4.0	3.5	.5

[1] The varietal names given to these 3 selections in order of their mention are Early Cheyenne 1, Cheyenne 2, and Cheyenne 3.

As the testing of the 74 selections progresses, it seems highly probable that some may prove superior to the 3 described. Furthermore, additional study of the segregating populations may bring to light plants superior to the selections already made. In selecting further, the breeder should give particular attention to the everbearing habit.

EVALUATION OF BREEDING METHODS

The question may be asked whether the broad method of breeding adopted, namely, that of hybridizing before inbreeding, was justified. The genetic disparity between species as regards the characters differentiating winter injury and size of fruit was so much greater than that within species as to render the latter negligible in accomplishing the principal objective of the breeding program. This fact alone justifies the adoption of the method employed. The same conclusion is warranted for plant vigor as represented by plant height, ability to produce runners, days from May 1 to first bloom, days from first bloom to first fruit ripe, and days from May 1 to first fruit ripe. Such, however, was not the case with the characters that influence quality, namely, sweetness, flavor, and firmness of fruit. However, in the central Great Plains and Rocky Mountain regions, quality as compared with winter hardiness is not a major problem. Therefore, it must be concluded that employing the broad method involving hybridization before inbreeding was fully justified.

Since hybridization before inbreeding was found to be the most promising method of obtaining immediate practical results, the next step was to determine which populations and lines of breeding offered the most promise. The F_1 hybrid of Fairfax $\times$ 37501 had the greatest number of plants combining extreme winter hardiness and fruits sufficiently large to be acceptable for home garden production. This was not true of the F_1 hybrids involving Fairfax and collections 36979 and 361477, a fact which shows that the frequency of occurrence of large fruit is also dependent upon the genotype of the collection of *F. ovalis*. Such being the case, further breeding programs should include the production and testing of a large number of F_1 hybrid plants involving the crossing of a number of collections with a number of cultivated varieties. The fundamental principle justifying such a procedure is that genes are present in some collections of *F. ovalis* which in the F_1 hybrid of crosses with $\times$ *F. ananassa* allow the almost complete if not the complete expression of the genes for large size of fruit. The only means of locating these collections of the native Rocky Mountain strawberry which carry such genes is by breeding tests such as those outlined above.

Backcrossing the F_1 hybrid to the cultivated parent has given very good results also. In fact, these results are even better than the data reported in table 6 would indicate. The reason for this is that the size of fruits falling in the desirable class will average larger for the backcross to Fairfax than will the F_1 fruits falling in this same class. Because of this fact, it seems that the backcross method probably offers the greatest possibilities for the most rapid accomplishment of the objectives of the breeding program. Further work therefore should involve backcrossing to the cultivated parent and also outcrossing to a number of the other cultivated varieties. However, in following the backcross and outcross methods of breeding, a more rigid selection of the F_1 plants should be made so as to obtain larger fruited F_1 plants than were available when the breeding program was started at Cheyenne. Also, some of the more promising selections from the backcross to Fairfax should be used in further backcrossing, in crossing among themselves, and in outcrossing to other cultivated varieties possessing special merit.

The double crosses are particularly promising because of the comparatively large proportion of the population possessing winter hardiness, because of the marked vegetative vigor of some of the double crosses, and because of the outstanding productiveness of some of the plants. Again large-fruited F_1 hybrids should be selected for making the crosses. If this is done there seems to be no reason why the plant breeder should not obtain extremely winter-hardy, large-fruited, and very prolific strawberries that would not only excel the present commercial varieties but also the selections already made from the breeding material involving the hybridization of ×*Fragaria ananassa* and *F. ovalis*. Also, outstanding double-crossed plants should be used in crossing among themselves and in outcrossing with existing varieties possessing special merit.

The F_2 population did not offer as much opportunity for selection as the other hybrid populations. This may have been partly due to the reduction of vigor in the F_2 population as compared with the F_1 hybrids and the double crosses. Table 5 shows that the size of fruits of the *Fragaria ovalis* collection 36979 inbred by self-pollination for one generation did not average as large as the fruits of the parent asexually propagated. The same was true of the fruits of the F_2 plants as compared with the fruits of the corresponding F_1 plants. Thus it would seem that when maximum segregation is desired double crosses offer far more promise than F_2 populations. This certainly was true in the studies reported herein. However, crossing among selected F_2 plants, outcrossing to meritorious existing varieties, and crossing with selections from other generations should produce selections superior to those now available.

BREEDING PRINCIPLES

The extremely infrequent occurrence of individuals recombining all the desirable characters is emphasized by the data for the double cross [F_1 (Fairfax × 36979) × F_1 (Dorsett × 37501)]. Assuming independent inheritance, to recombine all the desirable characters given in table 6 into a single individual would, on the average, require a population of 5 million plants. However, if the breeder is content with extreme winter hardiness, large fruit, exceedingly vigorous plants, ability to produce a large number of runners, and a date of maturity and quality of fruit similar to that of Dorsett and Fairfax, he may find such a plant on the average in every 100 plants of this double-cross population. This last statement explains why it was possible to obtain 74 promising selections from the breeding material involving crosses between the cultivated varieties and the native Rocky Mountain strawberry. The reason why so many plants, comparatively speaking, recombine these 4 desirable characters is that winter hardiness and ability to produce runners are almost completely dominant; larger size of fruit shows a small degree of partial dominance; and plant height shows a high degree of heterosis.

SUMMARY

This paper reports the results of breeding studies involving crosses between the cultivated varieties (× *Fragaria ananassa*) and the native Rocky Mountain strawberry (*F. ovalis*). The material included the parents asexually propagated, the progeny obtained by

selfing the parents, the progeny obtained by backcrossing to both parents, the F_1 hybrids, the F_2 population, and the double crosses.

The percentage of strawberry seeds germinating varied from 31.1 percent for selfed Fairfax to 96 percent for the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)]. The mean number of days required for the percentages of germination noted above was 69 for selfed Fairfax and 42 for the double cross.

Reciprocal crosses did not behave alike in respect to percentage or time of germination. The maternal parent was found to have a preponderance of influence. The percentage of germination was greatest and the time required for germination least when the F_1 generation was used as the female parent. In fact, the F_1 hybrid showed heterosis as regards this matroclinous effect.

The genetic disparity in the material indicates that by selection in segregating hybrid populations the breeder can build up breeding stocks that will show a high percentage of germination and that will germinate quickly.

The following characters showed either partial or complete dominance: A high degree of winter hardiness over winter injury; large over small size of fruit; a large over a small number of runners; a short over a longer period from May 1 to first bloom; and a short over a longer period from first bloom to first fruit ripe. Height of plant and the short period from May 1 to first fruit ripe showed heterosis. In both cases, this heterosis was retained by the double cross [F_1 (Fairfax $\times$ 36979) $\times$ F_1 (Dorsett $\times$ 37501)]. The data on quality of fruit (sweetness, flavor, and firmness) were not conclusive as regards dominance and heterosis.

The data on the frequency of the occurrence of plants possessing the more desirable of the contrasted characters showed that the opportunity for selecting the desired types was greatest among the plants of the backcross to Fairfax, certain F_1 hybrids, and the double crosses.

The studies on the frequency of the occurrence of plants recombining desirable characters show that only in the case of days from May 1 to first bloom and days from first bloom to first fruit ripe did genetic linkage reduce the number of plants combining the desirable characters; that is, the number of such plants obtained was less than the number expected if the genes differentiating the contrasted characters are independently inherited. In the other 13 cases of nonindependence of the characters, the relations found either helped in obtaining the desired combinations of characters or had no effect. In 31 of the possible 45 cases the characters were found to be independent.

On an average, 1 individual per 100 was found to recombine the following desirable characteristics: Extreme winter hardiness, large fruits, exceedingly vigorous plants, ability to produce a large number of runners, and a date of maturity and quality of fruit similar to those of Dorsett and Fairfax.

Seventy-four promising selections were obtained from the breeding material involving crosses between the cultivated varieties and the native Rocky Mountain strawberry. The reason why it was possible to obtain so many plants, comparatively speaking, that recombine the desirable characters is that winter hardiness and the ability to

produce runners are almost completely dominant, size of fruit shows a small degree of partial dominance, and plant height and short period from May 1 to first fruit ripe exhibit heterosis.

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ALFALFA SEED PRODUCTION AS AFFECTED BY ORGANIC RESERVES, AIR TEMPERATURE, HUMIDITY, AND SOIL MOISTURE[1]

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INTRODUCTION

In many parts of the world where alfalfa (*Medicago sativa* L.) is grown seed production is poor, whereas in other areas the seed comprises the major value of the crop. Climatic conditions are usually assumed to account for the differences. The object of the investigations reported here was to determine the extent to which organic reserves in the roots, soil moisture, and climatic factors, such as air temperature and relative humidity, affect seed setting. It is thought that external factors may influence seed production in alfalfa directly by affecting the development of top growth and the viability and growth of pollen and indirectly by affecting the internal factors of plant-food manufacture and storage. Experiments were started in 1934 at the Kansas Agricultural Experiment Station, Manhattan, Kans., to determine the importance of these factors and to ascertain whether certain treatments correlate with high seed production.

REVIEW OF LITERATURE

Many factors affecting seed development in plants have been studied. Hollowell (*9*)[2] stated that atmospheric humidity does not affect the setting of red clover seed in the greenhouse or field and that neither high nor low soil moisture prevents seed setting. However, he stated that plants grown with the soil moisture slightly below the optimum for growth matured flowers more satisfactorily for seed production.

Alter (*1*) stated that a certain amount of stress on the alfalfa plant was necessary to force the setting of seed. Observations have indicated that the production of alfalfa seed is best in areas where the air humidity is usually low and the soil moisture below optimum. Blinn (*3*) reported that good yields of beet seed were obtained by holding the irrigation water near the minimum requirement of the plant; he concluded that dry climatic conditions with high temperatures seem to be among the most essential requirements for seed production.

Carlson (*5*) stated that a desertlike climate, especially one with light but frequent summer rains, is considered a factor influencing

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[2] Italic numbers in parentheses refer to Literature Cited, p. 132.

alfalfa seed production in the valley lands of Utah. He also expressed the opinion that the frequent rains conditioned the atmosphere rather than the soil. Fuelleman [3] studied the effects of water utilization, organic reserves, and nitrogen supply on seed production in alfalfa and concluded that extreme retardation of vegetative vigor favored seed formation more than extreme acceleration of vegetative vigor. Nightingale and Farnham (*10*), working with sweet peas in the greenhouse, found that if water was used sparingly the salt concentration of the soil solution was increased, thereby limiting the absorption of water, inducing early maturity of tissue, and decreasing the rate of protein synthesis from nitrates. As a result carbohydrates accumulated, as they were used less extensively in protein synthesis. These authors stated further (*10, p. 512*): ". . . to obtain ideal vegetative and reproductive growth, the protein nutrition of plants must be considered in relation to opportunity for carbohydrate synthesis."

Garner et al. (*6*) noted the changes taking place in the carbohydrates in the upper parts of the stems of cosmos plants during short intervals. With Biloxi soybeans in the bud stage they found that reducing sugars increased during late summer and early fall, decreased when blossoms appeared, and increased again when seeds were developing. Soluble nitrogen also increased in the seed stage.

The period from the primordial stage of the alfalfa flower through the development of the seed may include 25 to 45 days. During that time the external factors may have changed several times, thus affecting the internal factors. Bolton and Fryer (*4*) noted such changes and stated that internal plant factors may not promote seed setting in alfalfa as definitely in the later stages as in the earlier stages of the flowering period.

From this partial review of the literature it is evident that external factors affect the seed production of certain plants and may do so in part by altering the internal factors. It is essential that more be known about the extent to which each factor affects production and how the external factors are related to the internal factors.

METHODS OF EXPERIMENTATION

Two controlled experiments were set up for these investigations, one for studying the influence of air temperature and relative humidity on seed production and the other for studying the influence of soil moisture and organic reserves. The chamber used for the control of air temperature and relative humidity, in which sulfuric acid was used to control humidity, was constructed and described by Grandfield and Zink (*8*). This equipment permitted the studies to be carried on in natural light under greenhouse conditions. To eliminate the inherent variability of different alfalfa plants, cuttings tracing back to a single plant were used each year in all experiments. The alfalfa plants were established in 6-inch clay pots and allowed to develop to the full-bloom stage. At the time the plants were to be placed in the chamber, the older flowers and buds were removed from each raceme, the remaining flowers were tripped and counted, and each raceme was tagged. In the course of the experiment different plants were exposed to various temperatures ranging from

[3] FUELLEMAN, R. F. WATER UTILIZATION AND OTHER FACTORS INFLUENCING THE FRUITING OF ALFALFA. Univ. of Wis. 2 pp. 1934. [Processed.

60° to 120° F. and to relative humidities ranging from 10 to 90 percent. It was learned from earlier trials that exposures of from 3 to 8 hours in the cabinet gave satisfactory results. After exposure the plants were returned to the greenhouse, where the seeds were allowed to develop. A record was made of the number of flowers tripped and of the pods and seeds formed.

The experiment on soil moisture and organic reserves was conducted by means of special equipment developed by the author (*7*) for this study. The soil was prepared by mixing five parts of Wabash silt loam and one part of sand with a small amount of compost. This mixture allowed water to penetrate readily and had a high water-holding capacity. The moisture equivalent and the wilting coefficient were determined for each experiment in the usual manner. The field water-holding capacity of the soil was determined by placing 12-inch columns of soil 1 inch in diameter in a perpendicular position on sand, saturating the soil with water, and allowing the columns to drain for 24 hours before determining the percentage of moisture. This percentage was assumed to represent the field water-holding capacity of the soil.

The experiment was conducted under two levels of soil moisture, referred to in this paper as "high" and "low." The high level was near the field water-holding capacity (22 percent); the low level was 12 percent, being 4 percent above the wilting coefficient. The watering was done as described by the author (*7*), and the pots were brought to the proper weight each day. The scales used permitted the pots to be weighed to an accuracy of ± 10 gm. One hundred pots were used in this experiment, with four combinations of soil moisture and food reserves as follows: Low moisture, low reserves; high moisture, low reserves; low moisture, high reserves; and high moisture, high reserves.

The high and low reserves were obtained by keeping the top growth cut back on plants in half the pots to reduce the reserves, and allowing the plants in the other pots to grow normally to the full-bloom stage. While the plants were becoming established and the reserves were being depleted in half of them, the soil moisture was held at 15 percent. After the plants had become established and the differences in reserves had been obtained, all the top growth was removed. Half of both the low-reserve and the high-reserve plants were reduced to the 12-percent soil-moisture level, and half were increased to the 22 percent level to obtain the four conditions just mentioned. As the plants came into bloom, each raceme was tagged and a record was made of the number of racemes and the number of flowers tripped, pods formed, and seeds developed.

Laboratory determinations for total carbohydrates were made by the iodometric method of Shaffer and Hartmann (*11*), and the amounts were calculated as dextrose from the Munson-Walker tables (*2*). Total nitrogen content was determined by the Kjeldahl method.

A soil-moisture-control and time-of-irrigation experiment was conducted by varying the amount of water supplied to the plants during seed-crop development and the seeding period. Four moisture treatments were used: (1) Low moisture; (2) low moisture from the time growth started on seed crop until prebud stage, with high moisture during the flowering and ripening period; (3) high moisture through-

out; and (4) high moisture until top growth was well started, low moisture until prebud stage, and then high moisture until seed was matured. High moisture was at 22 percent and low moisture at 12 percent, as in the previous experiment.

EXPERIMENTAL RESULTS

Preliminary work was started on the experiments in 1934, but, owing to the necessity of developing special equipment, definite results were not obtained until 1936. The investigations of the various factors affecting the production of alfalfa seed will be discussed under two headings, namely, Influence of Air Temperature and Relative Humidity, and Influence of Soil Moisture and Organic Reserves.

INFLUENCE OF AIR TEMPERATURE AND RELATIVE HUMIDITY

Air temperature, relative humidity, and soil moisture are closely associated in their interaction on crop plants, and conclusions cannot be drawn without taking all these factors into consideration. The combined effect of temperature and humidity under comparable soil-moisture conditions on the number of flowers setting pods is shown in table 1.

Under the conditions of this experiment very few alfalfa flowers set pods at temperatures above 100° F., regardless of the relative humidity. This is contrary to common observation, because alfalfa plants do set pods in the greenhouse where air temperatures are as high as 120°. The plants used were not hardened to high temperatures but were taken from the greenhouse bench where the air temperature ranged from 70° to 90°. Therefore, the maximum temperature for the proper functioning of the flower parts is lower than that for hardened plants. A supplemental experiment showed that alfalfa plants in the full-bloom stage could be hardened to high temperatures, so that they were able to set pods at temperatures as high as 120°.

TABLE 1.—*Effect of air temperature and relative humidity on percentage of alfalfa flowers setting pods*

Relative humidity (percent)	Flowers setting pods at indicated temperature (°F.) [1]							
	60	70	80	90	100	110	120	Average (60–110)
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
10	35	51	62	51	45	15	0	43
30	46	51	52	46	50	17	0	44
50	42	44	56	48	47	13	0	42
70	41	40	40	37	34	6	0	33
90	42	37	38	42	32	6	0	33
Average	41	45	50	45	42	11	0	

[1] Differences greater than 8.4 among the percentages, after arc sine transformation, are significant at 5-percent probability.

The data indicate that the greatest influence of humidity on the number of pods produced was exerted at a temperature of 80° F. At 110°, 6 percent of the flowers set pods under a relative humidity of 90 percent and showed a gradual increase to 17 percent as the humidity decreased. At temperatures from 70° to 110°, similar increases were

noted in the number of flowers setting pods as the humidity decreased. The average values show a decrease in pod setting at temperatures above and below 80°, with a marked decrease above 100°.

No significant differences were obtained in the average percentage of pods set at temperatures from 60° to 100° F.; but the decrease in pod setting was highly significant ($P < 0.001$) at 110°, and no pods were formed at 120°. The differences in the average number of pods set at relative humidities from 10 to 50 percent were not significant, but the reduction from the number set at the lower humidities (10 to 50 percent) to the number set at the higher humidities (70 to 90 percent) was highly significant ($P < 0.001$).

No significant differences were observed in the average number of seeds per pod at the different humidity levels or at the different temperatures from 60° to 100° F. (table 2). A highly significant reduction in seeds per pod occurred at 110° ($P < 0.001$), and, as previously noted, no pods formed at 120°.

TABLE 2.—*Effect of air temperature and relative humidity on the number of alfalfa seeds per pod*

Relative humidity (percent)	Seeds per pod at indicated temperature (°F.) [1]							
	60	70	80	90	100	110	120	Average (60–110)
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
10	1.8	1.8	2.1	2.0	2.1	0.8	0	1.77
30	2.3	2.1	2.1	1.4	1.8	1.0	0	1.78
50	2.0	1.7	2.0	2.2	2.1	.8	0	1.79
70	1.8	1.9	1.9	1.8	1.6	.7	0	1.61
90	1.6	1.7	1.8	1.9	1.8	.6	0	1.57
Average	1.9	1.8	2.0	1.9	1.9	.8	0	

[1] A difference of 0.83 in number of seeds per pod is significant at 5-percent probability.

INFLUENCE OF SOIL MOISTURE AND ORGANIC RESERVES

The response of alfalfa plants to the four combined treatments of soil moisture and organic reserves is illustrated in figure 1. In each treatment 25 pots were used, and as many as 19,000 flowers were counted in the higher seed-setting groups. Table 3 presents the data obtained, expressed as 4-year averages of the number of racemes,

TABLE 3.—*Effect of organic reserves and soil moisture on seed production in alfalfa*

Item	Average under conditions of—			
	Low moisture, low reserves	High moisture, low reserves	Low moisture, high reserves	High moisture, high reserves
	Number	*Number*	*Number*	*Number*
Racemes per plant	13	31	32	66
Flowers per plant	151	437	511	1,068
Pods per plant	87	247	271	550
Seeds per plant	174	526	568	1,101
Flowers per raceme	12	14	16	16
Pods per raceme	7	8	9	8
Seeds per raceme	13	17	18	17
Seeds per flower	1.2	1.2	1.1	1.0
Flowers per pod	1.7	1.8	1.9	1.9
Seeds per pod	2.0	2.1	2.1	2.0

flowers, pods, and seeds per plant and their ratios to one another. Each annual replication was made with the same soil mixture and with cuttings from the same plant.

FIGURE 1.—Effect of soil moisture and organic reserves on setting of alfalfa seed: *A*, High moisture, high reserves; *B*, low moisture, high reserves; *C*, high moisture, low reserves; *D*, low moisture, low reserves.

Under conditions of low moisture and low reserves, alfalfa plants produced an average of 13 racemes; whereas, under conditions of high moisture and high reserves, the plants produced an average of 66 racemes. There was a corresponding difference in the number of flowers, pods, and seeds formed. The number of racemes produced under high moisture and low reserves was approximately the same as the number produced under low moisture and high reserves.

The relative importance of soil moisture and food reserves in the production of alfalfa seed under the four conditions is shown in table 4. Under the low-moisture condition, the high-reserve plants produced 146 percent more racemes per plant than were produced by the low-reserve plants. With high moisture, the increase due to higher

reserves was only 113 percent. Similar increases due to high reserves were obtained in the number of flowers, pods, and seeds produced. High moisture produced the same trend under the conditions of low and high reserves. With low reserves, the high moisture increased the production of racemes 138 percent over the low moisture; and with high reserves, the high moisture increased the number of racemes 106 percent.

TABLE 4.—*Effect of soil moisture and organic reserves on increase of alfalfa racemes, flowers, pods, and seeds per plant*

Item	Increase, due to high reserves, over low reserves at—		Increase, due to high soil moisture, over low soil moisture at—	
	Low moisture	High moisture	Low reserves	High reserves
	Percent	*Percent*	*Percent*	*Percent*
Racemes	146	113	138	106
Flowers	238	144	189	109
Pods	212	123	184	103
Seeds	226	109	202	94

Root samples were taken for carbohydrate and nitrogen analyses to determine the actual amount of organic reserves in the roots, and soil samples were also taken to determine the percentage of soil moisture maintained. The data in table 5 show that, on the date the seed crop started to develop, the low-reserve plants contained less total carbohydrates than the high-reserve plants. This condition existed until the last sampling date. By that time the low-reserve plants were able to store carbohydrates and build up a reserve. The low-reserve plants in 1942 were much lower in total carbohydrates at the first sampling date than the corresponding plants in 1941, because of the severer treatment they received before they were sampled.

TABLE 5.—*Percentage of soil water and of organic reserves in roots taken from pot samples*

Top growth development	Moisture	Reserves	Soil water	Total carbohydrates		Total nitrogen	
				1941	1942	1941	1942
			Percent	*Percent*	*Percent*	*Percent*	*Percent*
None	Low	Low	12.6	42.2	26.6	1.2	3.0
	High	do	21.9	38.4	19.4	1.1	3.3
	Low	High	12.4	49.8	41.9	2.0	3.0
	High	do	22.1	45.1	41.0	2.1	3.4
8 inches	Low	Low	12.0	43.8	22.5	1.5	3.1
	High	do	18.5	45.1	20.0	1.3	3.4
	Low	High	11.7	50.9	37.5	1.7	3.1
	High	do	20.0	50.0	35.4	1.7	3.2
Seed stage	Low	Low		42.2	36.4	1.2	3.4
	High	do		52.3	37.2	1.8	
	Low	High		53.9	37.3	2.1	3.0
	High	do		50.0	38.0	2.4	2.7

The average soil-moisture content of the low-moisture pots was 12.2 percent and that of the high-moisture pots 20.6 percent (table 5); these values are very near the desired percentages of 12 and 22, respectively.

The data on the effect of amount and time of irrigation on seed production are reported in table 6. The 2-year averages show little difference in the number of seed produced under the different treatments, except in the low-moisture pots. The plants given the low-high moisture treatment produced an average of 81 racemes per plant, which was 9 less than that produced by the continuous high-moisture treatment. There was a corresponding difference in the number of flowers, pods, and seeds formed. The ratio of flowers to racemes in the low-high pots was 15 to 1, which was the highest ratio for any of the treatments. Although the differences were small for the three treatments, it was evident that a low-moisture condition for a part of the time during the growth of the seed crop is not detrimental to seed production.

TABLE 6.—*Effect of amount and time of application of irrigation water on alfalfa seed production*

Item	Average under indicated treatment			
	Low moisture	Low-high moisture [1]	High moisture	High-low-high moisture [2]
	Number	*Number*	*Number*	*Number*
Racemes per plant	41	81	90	85
Flowers per plant	545	1,235	1,247	1,179
Pods per plant	230	526	547	510
Seeds per plant	385	868	866	770
Flowers per raceme	13	15	14	14
Pods per raceme	6	6	6	6
Seeds per raceme	9	11	10	9
Seeds per pod	1.7	1.7	1.6	1.5

[1] Low moisture from start of seed-crop growth until prebud stage, and high moisture during flowering and ripening period.
[2] High moisture until top growth was well started, low moisture until prebud stage, and high moisture until seed matured.

DISCUSSION

Previous investigations have shown the importance of the relation between the organic reserves in the roots and the vigor of the alfalfa plant. In the investigation reported here, it is shown that organic reserves stored in the roots are important also in relation to the production of seed. Air temperature, relative humidity, and soil moisture all affect the growth of the plant and thereby affect the storage of organic reserves.

The data from experiments in which temperature and humidity were controlled show the direct effect of these factors on the functioning of the reproductive parts of the plants. A statistical analysis of the data given in table 1 shows significant differences in the percentage of flowers setting pods as affected by temperature and humidity. The most favorable temperature was 80° F., and humidities above 50 percent were definitely detrimental to seed production. These data also indicate that the high humidity is more detrimental at 110° than at lower temperatures.

Soil moisture has a marked effect on the nature and rapidity of growth, thereby affecting the manufacture and storage of organic reserves. Any factor that influences the vigor and type of plant growth, thereby influencing plant-food manufacture and storage, may have a direct effect on seed production.

The data presented in table 5 show that in the low-moisture plants the values for total carbohydrates were generally slightly higher than those in comparable high-moisture plants, indicating that low moisture was conducive to a slower rate of top growth and thus enabled the plants to manufacture food faster than it was being used. This condition existed until the seed stage, when the top growth was of sufficient quantity and maturity in all treatments to build reserves. This development brought about the conditions described by Nightingale and Farnham (*10*), who stated that carbohydrate synthesis is important in obtaining ideal reproductive growth in sweet peas. In the author's experiments, less vigorously growing alfalfa, with high reserves, produced more racemes, flowers, pods, and seeds than plants with low reserves under comparable soil-moisture conditions (table 3). In the plants forced to grow under stress caused by external factors, a physiological situation was established favoring seed production when organic reserves were plentiful. These data show also that increased soil moisture had its greatest effect on plants under the less favorable condition of low reserves. The combination of high reserves and high soil moisture showed the highest production of seed in this experiment. However, preliminary tests indicate that this combination may not give the same results under field conditions where the plants are closely spaced, as the excess top growth and crowding would appear to retard blooming. In this greenhouse experiment each plant had a restricted space for root growth but adequate space for the maximum light required for branching and flowering.

Data for amount and time of irrigation (table 6) indicate that the low-high-moisture treatment brought about a condition during the early growth period favorable for the conservation of carbohydrates, and the delayed application of water made it possible for the energy stored in the form of carbohydrates to be used for seed production. This treatment produced the highest number of flowers and seeds per raceme. While the differences were not great, they were produced on a smaller number of racemes, indicating that the low-high-moisture treatment was less favorable for the production of racemes but more favorable for pollen germination and seed development.

SUMMARY AND CONCLUSIONS

A study has been made to determine the extent to which organic reserves in the roots, soil moisture, and climatic factors affect seed production in alfalfa.

Under the conditions of this experiment the number of alfalfa flowers setting pods decreased at temperatures above 100° F. The optimum temperature for pod setting was 80°.

The number of flowers setting pods increased as the relative humidity decreased from 90 to 10 percent. At 80° F., the difference between the percentage of pods set at 10 percent relative humidity and the percentage set at 90 percent was 24.

High reserves increased seed production; the greatest increase occurred when moisture was low. When the reserves were low, seed production was greater when the soil moisture was high.

Moderate air temperature, low humidity, and soil moisture below optimum produced the type of vegetative growth of alfalfa plants that

was conducive to the storage of high organic reserves, resulting in a physiological condition favorable to seed setting.

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RELATION OF MACROFUNGI AND MICRO-ORGANISMS OF SOILS TO DAMPING-OFF OF BROADLEAF SEEDLINGS[1]

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INTRODUCTION

At present the only reliable method of determining the damping-off potentialities of untried nursery soils is to ascertain the damping-off of susceptible seedlings in greenhouse or field trials. The object of the study reported here was to determine whether there is any relation between the abundance of macrofungi and micro-organisms in the soil and the damping-off of broadleaf seedlings.

MATERIALS AND PROCEDURE

In the first part of this study the macrofungi growing on Marshall silt loam in field and greenhouse tests were identified and the associated damping-off losses were compared for such broadleaf seedlings as *Ulmus americana* L., *U. pumila* L., *Robinia pseudoacacia* L., and *Chilopsis linearis* (Cav.) Sweet.

Dilution-plate counts were made to determine the number of fungi and bacteria in sandy loam and silt loam. The micro-organisms were studied also by the use of modified Cholodny soil slides. The resulting data were compared with the damping-off losses occurring on the same soils in crocks kept in the greenhouse. These tests were made at Lincoln, Nebr., from 1937 to 1939.

MACROFUNGI AND DAMPING-OFF

It has been observed in both field and greenhouse tests that the sporophores of certain fungi commonly follow specific agronomic crops.

One of the commonest sporophores found was that of the bird's-nest fungus, *Cyathus vernicosus* DC. It occurred most frequently in soil where corn had been grown or where wheat had followed corn. The base of the sporophore was generally attached to a piece of buried cornstalk. These sporophores have been observed only in the fall after rather heavy rains. In uncultivated fields they sometimes occur also in the spring, but the indications are that cultivation disturbs the soil to such an extent that it interferes with sporophore formation. What part, if any, this fungus plays in damping-off is

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[2] This study was begun under the direction of Dr. George L. Peltier, of the Department of Bacteriology, and continued under the supervision of Dr. Robert W. Goss, of the Department of Plant Pathology, University of Nebraska. The writer gratefully acknowledges his indebtedness for this guidance. He also wishes to express his thanks to Floyd R. Schroeder for the valuable assistance rendered in obtaining the photomicrographic pictures presented here. Greenhouse and laboratory equipment was provided by the Department of Plant Pathology of the University of Nebraska.

unknown. However, greenhouse tests have indicated that soils on which the sporophores of *C. vernicosus* occurred were favorable to the broadleaf seedlings tested, since damping-off losses in these soils were relatively light.

Sporophores of *Naucoria semiorbicularis* Fr. have often been observed in fields where gramineous crops have been grown in successive rotations. They were particularly abundant in soils where wheat followed wheat or where rye followed wheat. On rye plots at the Nebraska State Agricultural Farm, these sporophores were observed to follow each drill row. Examinations showed that rhizomorphs extending from the sporophores were frequently attached to the bases of the plants or to their roots. However, Sprague (*12*) [3] found that closely related agarics were not pathogenic to cereals. Kauffman (*8*) also indicated that *N. semiorbicularis* is not pathogenic to grasses. Greenhouse tests showed that damping-off losses were heavy on such soils, and the abundance of these sporophores indicates that the soil is unfavorable for susceptible broadleaf seedlings.

Species of *Coprinus* observed both in the field and in potted greenhouse soils were especially common following alfalfa. Damping-off losses on such soils were heavy. The abundance of *Coprinus* sporophores indicates that such soils favor damping-off of broadleaf seedlings.

SOIL MICRO-ORGANISMS AND DAMPING-OFF

It was early recognized by pathologists that meteorological factors and environmental conditions have a strong influence on damping-off (*2*). They likewise affect the development of the soil micro-organisms (*11*). The number of micro-organisms in a soil is known also to be directly related to the depth, texture, and moisture content of the soil, the amount of organic matter present, the acidity, etc. (*14*). With so many complicating factors involved, it was recognized at the beginning of this investigation that in the time available a complete study of soil micro-organisms in relation to damping-off could not be made.

Probably the best method of determining the damping-off fungi present in the soil is by direct isolation from diseased seedlings and reinoculation to prove pathogenicity. In a previous paper (*17*) it was shown that *Rhizoctonia solani* Kühn and *Pythium ultimum* Trow are the principal fungi causing damping-off of broadleaf seedlings in Great Plains nurseries. Other micro-organisms in the soil may also be important.

DILUTION-PLATE STUDIES

METHOD

In preparing soils for dilution-plate studies, moisture contents were first determined on an oven-dry basis. After these differences had been taken into consideration, equivalent 10-gm. samples were placed in 100-cc. sterile water blanks and shaken vigorously for 2 minutes. Dilutions of 1:1,000 in peptone-glucose agar were used for the fungi. Bacteria were cultured on sodium albuminate agar at a dilution of 1:10,000 (*6*).

Dilution plates were poured in quadruplicate, and final counts of fungi were made after 4 days' incubation at 25° C., and of bacteria after 7 days' incubation.

[3] Italic numbers in parentheses refer to Literature Cited, p. 140.

Results

These studies were repeated several times in conjunction with greenhouse damping-off tests, but a consistent relation has never been demonstrated between damping-off losses of broadleaf seedlings and the number of fungi and bacteria present in dilution plates. In general it appeared that the greater the number of fungi in the soil the heavier was the damping-off, whereas the reverse was generally true for bacteria except in soils occupied by legumes. It seemed possible that species of fungi present in the dilution plates might be important, but studies to obtain information on this point have also given negative results. Admittedly, studies to determine the relation of damping-off to the number of micro-organisms in the soil should be carried on for several successive years; but such studies were attempted only in a preliminary way in the present investigation. Whether a more complete study would be of greater practical value remains to be determined.

The kinds of fungi commonly appearing in the dilution plates were as follows: A yellow *Aspergillus* sp., *Trichoderma lignorum* (Tode) Harz, *Fusarium* spp. (both pink and white forms), *Rhizopus* spp., *Pythium* spp., *Mucor*, certain unidentified species of the Phycomycetes, and an unidentified white fungus. Of all these fungi, the yellow *Aspergillus* seemed to be the most prevalent. This fungus gave the medium adjacent to its hyphae a yellowish color.

No relation was found between the abundance of *Trichoderma* and damping-off losses, as reported by Weindling and Fawcett (*15*) for citrus seedlings. However, this is not regarded as important negative evidence, since the present investigations were made under conditions entirely different from those reported by Weindling and Fawcett. *Trichoderma* was much less abundant in these prairie soils than the other fungi named in the preceding paragraph.

While dilution-plate counts were used to determine the more common soil micro-organisms, such as the molds and bacteria, the microslide technique originated by Cholodny (*3*) was also employed to determine the micro-organisms in localized portions of the soil. Both methods have serious drawbacks. In dilution plates an approximation of the numbers of spore-forming fungi present in the soil can be obtained, but important non-spore-forming fungi, such as *Rhizoctonia*, are rarely isolated by this method. Thus dilution-plate counts may actually prove to be misleading for some purposes. The Cholodny method was used to identify the micro-organisms of the soil adjacent to plant roots, but the method cannot be used quantitatively. However, a modified Cholodny technique proved somewhat more useful.

MODIFIED CHOLODNY SOIL-SLIDE STUDIES

Method

In the present study, sterilized glass microslides were placed in sterile Petri dishes and coated with malt agar.[4] As soon as the agar had hardened, the slides were removed aseptically and slowly dried on an electric plate at 45° to 50° C. until the agar was reduced to paper thinness. These dried agar slides were inserted endwise into potted soils containing seedlings from which damping-off counts were

[4] Malt agar was found to be especially desirable for general tests, principally because it adheres very firmly to the slide after drying.

being obtained in the greenhouse.[5] First a hole about 2 inches deep was made by inserting a spatula into the soil and pulling it backward so that one face of the hole remained smooth and flat. Then the dried agar surface was placed against the flat side of the hole, and the loosened soil was packed down to hold the slide firmly in place.

These slides were left in the soil for 3 to 4 days. During this time the dried malt agar absorbed enough moisture from the soil to become softened, and thus furnished a suitable substratum for the growth of micro-organisms. Finally, the slides were extracted from the soil by pulling the agar-coated surface away from the flat side of the hole. After removal, the slides, with the agar surface up, were placed in Petri dishes and brought to the laboratory, where they were examined with a binocular microscope. In this way it was sometimes possible to detect individual colonies of fungi or bacteria, from which isolations were made for identification purposes. Ultimately the slides were slowly heated until the agar was again dried to paper thinness; then they were stained for detailed examinations. Best results were obtained by staining the dried agar slides with a rose bengale solution as described by Conn (*5*). After staining, the slides were ready for observation under high-power magnification. These stained, dried agar slides have been kept for several years, and they are still good for microscopic study even with an oil-immersion lens.

Results

In slides observed under a binocular microscope before drying, it was sometimes possible to detect the movements of nematodes and an occasional amoeba on the surface of the agar. In some cases the nematodes seemed to be feeding on the fungi, particularly on the fungus hyphae.

After examining dozens of stained, dried agar slides, the writer found no evidence of antagonism between fungi, between bacteria, or between fungi and bacteria, although actinomycetes appeared to develop best in less densely populated areas of the slides. As Conn (*5*) had found, bacteria were most abundant in soils of high moisture content. The species of *Aspergillus* that caused a yellowish discoloration in the agar dilution plates was also frequently observed on the dried agar slides.

In soils with abundant nematodes, there appeared to be a relation between the number of nemas on the dried agar slides and the kind of rotation crop. In these tests, nematodes were found to be more numerous in soils obtained from cereal plots than in those from leguminous rotations. Other tests had already indicated that the use of most legumes as preceding crops increased damping-off of broadleaf seedlings. The relation of damping-off to the number of nematodes is interesting, since Christie and Arndt (*4*) were first inclined to believe that nemas supposed to be saprophytic may cause root troubles of cotton seedlings. They later concluded (*1*) that their experiments failed to prove that the species of nematodes investigated were factors of primary importance in the etiology of damping-off, or sore shin, in cotton. However, Wilde (*16*) considered that nematodes were directly connected with damping-off losses in coniferous nurseries in

[5] The modified Cholodny slide method, which was developed during the course of these studies, was later found to be almost identical with that previously described by Kriuchkova (*9*).

Wisconsin. It is apparent that the observations presented here, which indicated that soils with numerous nematodes have less damping-off than soils with smaller populations, need confirmation.

Figures 1 and 2 show photomicrographs of stained, dried malt-agar slides, which illustrate some of the more common micro-organisms observed by the use of the modified Cholodny technique.

Pythium, other unidentified species of Phycomycetes, *Rhizoctonia*, *Fusarium*, *Penicillium*, and many other fungi were readily identified on these stained, dried agar slides.

Likewise of interest was the fact that the modified Cholodny slides showed clearly the initial stage of sclerotial formation of *Rhizoctonia*. It appeared that where *Rhizoctonia* hyphae came in contact with a solid object, such as the glass slide, they flattened out considerably prior to sclerotial formation.

Since this modified Cholodny technique satisfactorily showed the presence of bacteria and actinomycetes, as well as spore-forming and non-spore-forming fungi, besides some forms of microfauna, it is regarded as a valuable supplement to soil-dilution studies in determining the nature of the microbial population of the soil.

The Cholodny technique is applicable to both field and greenhouse conditions. By using selective agar it is possible in some instances to study the development of specific organisms while nearly excluding others. However, if specialized agar is used, preliminary tests indicate that malt sugar should be included in the medium, since this carbohydrate when heated has a marked tendency to cause the agar to stick firmly to the slide.

A method developed by Canadian workers (*7*, *13*) has enabled them to determine the micro-organisms present within the roots of certain plants, but it is not regarded as applicable to the present study. No attempt was made to make comparative tests by direct microscopic examination of the soil as described by Kubiena and Renn (*10*).

DISCUSSION

From the foregoing preliminary results, it does not appear likely that a thorough and more complete study of the microbial population of the soil would furnish a satisfactory clue to the damping-off potentialities of untried nursery sites. In such studies it would be necessary to include many soil types as well as different crop sequences, and the tests should extend over several years so that the effects of meteorological factors could be determined. Such studies are complicated by the fact that climatic variations affect the host, the activity of the microbial population of the soil, and, over a period of years, the soil itself. Whether these effects run parallel, run in opposite directions, or have any relation at all is at present not definitely known. In the final analysis, standards should be established on the basis of definite numbers of micro-organisms and should not be merely comparative. Assuming that such a standard could be obtained, it is doubtful whether it would be of much value to field technicians, since the technique required to make it dependable would probably be so refined as to preclude practical use.

At the present time it appears that the best method of predetermining the damping-off hazard of an untried soil is by greenhouse tests. However, microbiological studies are of considerable scientific interest

FIGURE 1.—Dried agar-soil slides stained with rose bengale (modified Cholodny slide technique). *A*: *a*, Beginning of sclerotial formation of *Rhizoctonia solani*; *b*, hyphae of other fungi; *c*, a nematode. ×118. *B*: *a*, Soil particles; *b*, *Actinomyces*, hyphae; *c*, a nematode. ×118. *C*: *a*, Hyphae of various soil fungi; *b*, nematodes; *c*, *Pythium* sp., oospore. ×233. *D*: *a*, *Phycomycete*, hyphae; *b*, *Penicillium*. ×233.

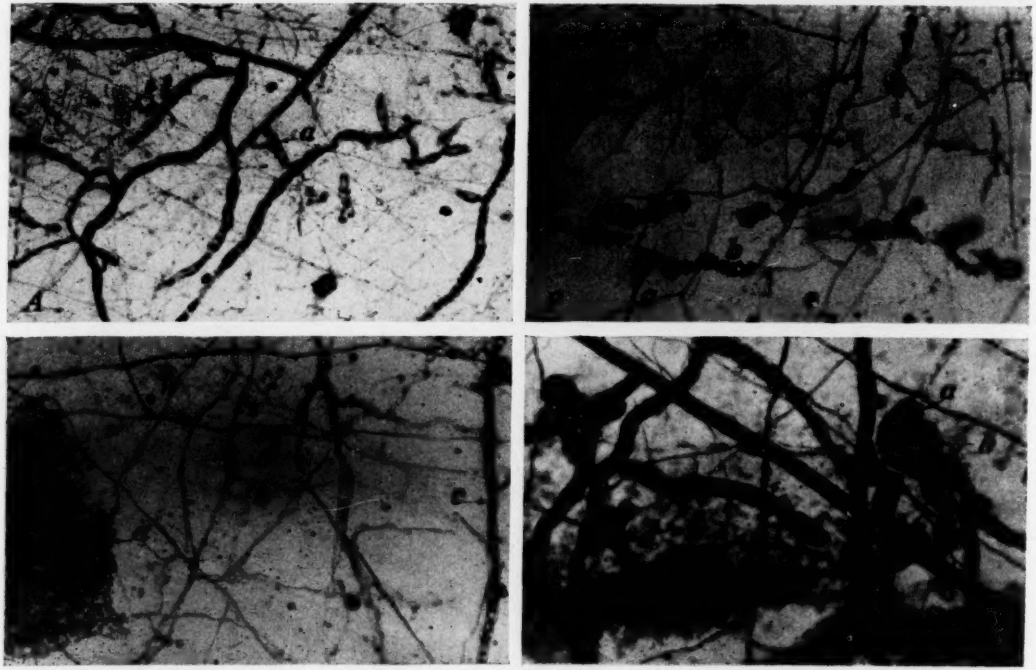

FIGURE 2.—Dried agar-soil slides stained with rose bengale (modified Cholodny slide technique). *A: a, Fusarium* sp.: *a,* Hyphae above other mycelial strands. ×233. *B, Actinomyces: a,* Hyphae; *b,* spores. ×233. *C: a,* Bacterial colony; *b, Actinomyces,* hyphae; *c, Fusarium,* hyphae. ×560 *D, Pythium* sp., hyphae showing (*a*) antheridium and (*b*) oogonium. ×560.

if not of practical value, and in special cases where numerous treatments are being tested on the same soil such studies may prove useful in determining variations between individual plots or treatments.

SUMMARY

Observations on the occurrence of soil macrofungi were made, and an apparent relation was noted between the occurrence of such fungi and the damping-off of broadleaf seedlings. The occurrence of sporophores of *Cyathus vernicosus* was associated with soils favorable to the growth of broadleaf species and low incidence of damping-off. An abundance of sporophores of *Naucoria semiorbicularis* and *Coprinus* spp., on the other hand, was associated with soils in which damping-off was high.

Soil micro-organisms were studied by dilution-plate counts and by a modification of the Cholodny microslide technique. The results obtained by the former method were not consistent. The general trend indicated that the greater the number of fungi present in the soil the heavier was the damping-off loss. However, soils containing the largest number of bacteria generally showed the least damping-off, except for sites on which leguminous crops preceded the broadleaf seedlings.

In a modified Cholodny technique, dried agar-coated slides were inserted into the soil to furnish a medium for sporulating and non-sporulating fungi, bacteria, and other micro-organisms. Nematodes were frequently observed in abundance; these appeared to be most numerous in soils on which cereal crops had been grown and on which damping-off losses were relatively light. The modified Cholodny slide technique used is believed to be of value for making a detailed study of the microbial population of the soil.

On the basis of the data presented, it does not appear likely that detailed microbiological studies of the soil will prove of practical value in predicting damping-off losses.

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